Variation in parasitism of intertidal invertebrates, with a focus on trematodes on the southwest of Ireland

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Declaration

This thesis has not been submitted for another degree either at University College Cork or elsewhere. The thesis is my own work and any aspect of the work contributed by colleagues is specified in the acknowledgments. This thesis may be available for consultation within the university library.

Signed: 

Amy-Ciara Geraghty

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GENERAL ABSTRACT

Parasites are ubiquitous in marine ecosystems, are sensitive to a range of environmental conditions, share characteristics of traditional bioindicators, and can be indicators of environmental stress. To investigate the role of environmental stressors on parasite communities, specifically trematodes, parasitological surveys of marine invertebrates were conducted across spatial and temporal scales on the coast of Ireland. The potential impact of stressors on parasite abundance, diversity and ability to infect new hosts was investigated.

The response of parasite communities and trematodes to environmental stressors varied in relation to habitat, stressor, host and parasite taxa surveyed. Water quality classifications were defined by the Water Framework Directive and used as a proxy for environmental stress. From May 2014 to April 2015, parasite communities of the common periwinkle *Littorina littorea,* the dogwhelk *Nucella lapillus* and the common limpet *Patella vulgata*, were sampled on three shores of differing water classifications and Lough Hyne Marine Reserve, Co. Cork. Large variation in parasite richness and abundance was observed between gastropod species, months and sites sampled. Higher occurrence and intensities of trematode infections in *P. vulgata* and *L. littorea* were associated with the site of good water quality. From June to August 2015, the Ragworm, *Hediste diversicolor,* and green shore crab *Carcinus maenas* were surveyed in three estuaries of differing enrichment levels. Occurrence and intensities of trematode taxa were recorded in *C. maenas* and *H. diversicolor* in the site of lower water quality. The increased abundance of trematodes, higher concentration of nutrient parameters and larger bird communities recorded, suggested that eutrophication may encourage trematode infection.

The potential of statutory protection, to encourage parasite communities was assessed by a yearlong survey (May 2013-April 2014) of subtidal *Mytilus* spp. populations and a short survey (June 2015) of intertidal *P. vulgata* populations in and around Lough Hyne. Parasite communities of both invertebrate taxa were similar in abundance and composition within and outside of Lough Hyne marine reserve. The limited exchange between outer waters and Lough Hyne, suggested that the reserve is unlikely to facilitate parasite taxa abundance. To investigate the observed low prevalence of trematodes in subtidal *Mytilus* spp., a series of laboratory trials was undertaken to assess the role of host stress on susceptibility to trematodes. However, the study was inconclusive due to a high level of natural trematode infection.

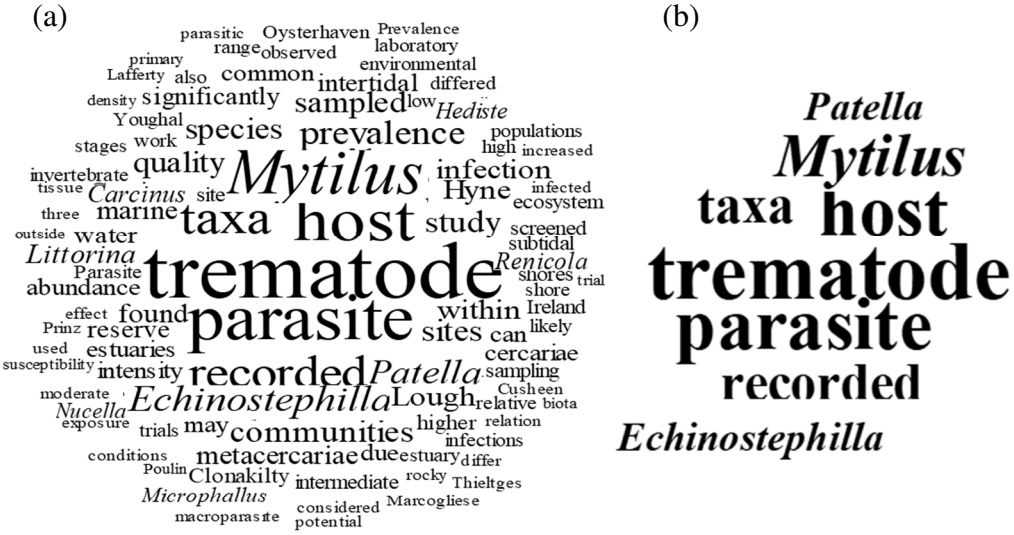

Fig. 1 Word clouds generated from the following chapters, excluding citations and references, with (a) words with a minimum frequency of 10 and (b) a minimum frequency of 100. The size of a words indicates their frequency of occurrence with larger sizes representing words with a higher frequency.

CHAPTER 1: General Introduction

The oceans cover the majority of this planet and contain an estimated ~2.2 million species (Mora *et al.,* 2011). Yet before 91% of these marine species can be described, they are being lost with mainly unknown consequences (Mora *et al.,* 2011). Marine biodiversity is lost at increasing rates and can be measured in extinctions, population depletions and community homogenisation (Sala *et al.,* 2006, Worm *et al.,* 2006, Crowe *et al.,* 2015). This loss is largely due to the widespread impacts of human activities on the marine environment; to the extent that no area has been left unaffected (Halpern *et al.,* 2008, Crowe *et al.,* 2015). The impacts can be as a result of a combination of stressors, such as coastal development, overexploitation, pollution and climate change (Jackson *et al.,* 2001, Halpern *et al.,* 2008). The loss of biodiversity impacts on the functioning of marine ecosystems and their ability to provide ecosystem goods and services, on which society depends (Duarte 2000, Hooper *et al.,* 2005, Worm *et al.,* 2006, Beaumont *et al.,* 2007). These ecosystem goods and services are thought to be best supported by diverse biological communities (Worm *et al.,* 2006). Consequently, biodiversity loss may jeopardize future generations' food security, livelihoods and safety, which can have implications for a growing population of 7 billion and especially for the estimated 1,200,000,000 people living within 100km of the coast (Small *et al.,* 2003). Society's health could be affected as changes may lead to increases in the occurrence and severity of disease or parasitic infection (Ward & Lafferty, 2004, Ostfeld & Keesing, 2000).

Marine ecosystem health (MEH) is a concept created to illustrate the state of the marine ecosystem (Constanza 1992, Marcogliese, 2005). MEH has attracted increasing scientific interest in the past decade (e.g. Halpern *et al.,* 2012). Health has been reported to be an easily understood, but poorly suited analogy for the state of a

marine ecosystem (Constanza 1992, Rapport *et al.,* 1998, Belaoussoff *et al.,* 2003). This is because (a) ecosystems are not organisms regulated by physiological processes, (b) the ambiguity of the term "health" and (c) the difficultly in attaining a quantifiable measure of "health" (Wicklum *et al.,* 1995, Belaoussoff *et al.,* 1998). MEH has varied definitions in the literature, ranging from the wellbeing of a coupled human-natural system or a reflection of the condition of the marine environment from the perspective of adverse effects caused by anthropogenic activities (Constanza 1992, Knap *et al.,* 2002, Strain *et al.,* 2002, Halpern *et al.,* 2012). Furthermore, MEH is utilised synonymously with ecosystem integrity, stability, functioning or ecological quality in the literature (Muniz *et al.,* 2011). Assessing whether an ecosystem is "healthy" is difficult in relation to assessing whether it is impacted or not by anthropogenic activities. This is carried out on a case-by-case basis or by inferring the status of an ecosystem from the state of sentinel organisms (Bremner *et al.,* 2003, Well, 2003). The majority of MEH assessments, and this thesis, adopt the latter approach.

Sentinel organisms or environmental indicators are taxa or communities which often reflect environmental conditions. Changes in the physiology, behaviour or presence of sentinel organisms or composition of communities within an ecosystem can indicate stress in an ecosystem (Goodsell *et al.,* 2009). Sentinel organisms or indicators can increase the generality of findings and the speed at which declines in MEH are detected. Molluscan communities have been commonly used as sentinel taxa as they are well studied, have wide geographic distributions and are abundant in marine ecosystems (Stewart *et al.,* 2008, Goodsell *et al.,* 2009, Atalah & Crowe, 2012). Mussel species have been preferentially utilised as sentinel organisms on a global scale due to the taxon's wide geographic distribution, abundance, persistence, sedentary nature, and ability to concentrate metals and organic contaminants (Hellou *et al.,* 2003, Kim *et al.,* 2008, Marigómez *et al.,* 2013, Brenner *et al.,* 2014). In particular, *Mytilus*

spp. have been widely used in large biomonitoring program e.g. “Mussel Watch”, to investigate regional patterns in ecosystem health in relation to heavy metals, pollution and parasites (Goldberg, 1975, Kim *et al.,* 2006; 2008). Other studies have considered an index, a health status index (HSI) which has been used successfully to track the health of *Mytilus* spp. after the Prestige Oil Spill and to assess levels of pollution along Greek coasts (Raftopoulou & Dimitriadis 2010, Marigómez *et al.,* 2013). Pollution biomarkers in the digestive gland of *Mytilus galloprovincialis* and HSI collected revealed high stress conditions in locations sampled (Raftopoulou & Dimitriadis 2010). HSI and Bioeffects Assessment Index (BAI) were among the five integrated biomarker indexes used in Galicia and the Bay of Biscay to simplify the interpretation of ecosystem stress in relation to the pollution of the marine habitats (Marigómez *et al.,* 2013). Gastropods have also been utilised as sentinel species. The common periwinkle *Littorina littorea* (Linnaeus, 1758) and the dogwhelk, *Nucella lapillus* (Linnaeus, 1758), have been commonly used in the detection of environmental tributyltin (TBT) by recording the occurrence of intersex and imposex in the prosobranchs (e.g. Gibbs *et al.,* 1987, Harding *et al.,* 1992, Oehlmann *et al.,* 1998). The tissue and radula of the common limpet *Patella vulgata* have been used to monitor heavy metal pollution in the Atlantic (Miramand and Bentley, 1992, Davies *et al.,* 2005). The Ragworm *Hediste diversicolor* (OF Müller, 1776) has been used as a sensitive bioindicator of heavy pollution (Scaps, 2002, references therein). The Green shore crab *Carcinus maenas* (Linnaeus, 1758) has been used to test the impact of toxicants and as a bioindicator in the monitoring of heavy metal contaminants (Rodrigues & Pardal, 2014).

The response of sentinel organisms to stressors or anthropogenic impacts can be altered by parasites, which are organisms dependent on, or that benefit from another organism, a host (Rohde, 2002, Lefevre *et al.,* 2009). In molluscs, trematode infection

has resulted in castration, endocrine disruption and increased mortality in hosts. When trematode infection and pollution co-occur, decreased respiration, metabolism, food consumption and cardiac activity has been recorded in bivalves (Morley, 2010). Multiple reviews call for the need to include parasites in pollution studies. Parasites can alter the host's response to a pollutant or accumulate the pollutant in its own tissues (Marcogliese & Pietrock, 2011, Sures *et al.,* 2017).

Parasites as indicators of MEH

Parasites are initially a counterintuitive measure of ecosystem health. However, parasites have many of the characteristics of more traditional sentinel organisms. Parasites are ubiquitous, considered to be the most common lifestyle on earth, and can be highly sensitive to environmental conditions (Mouritsen and Poulin, 2002, Clausen *et al.,* 2008, Dobson *et al.,* 2008). Changes in environmental conditions may affect the complex host-parasite relationship. Parasite infection may increase, if host abundance or susceptibility increases, or decrease, if the infected hosts experience increased mortality rates (Sures *et al.,* 2017). Parasites with complex lifecycles, free living stages or that intermittently survive external to the host, may parallel the response of free living biota, as both are in direct contact with the environment (Mackenzie, 1999, Pietrock and Marcogliese, 2003, Vidal-Martinez *et al.,* 2010, Aguirre-Macedo *et al.,* 2011, Sures *et al.,* 2017). In aquatic environments, the free living stages of parasitic worms, helminths, are known to be sensitive to a range of abiotic factors such as, temperature, salinity, light, pH, water pressure, and surrounding biotic communities (Pietrock and Marcogliese, 2003, Thieltges *et al.,* 2008, Welsh *et al.,* 2014). Parasite mediated effects are recognised to influence a range of ecosystem functions and food web structures (Lafferty, 1997, Marcogliese, 2005, Thompson *et al.,* 2005, Hudson *et al.,* 2006). From an ecological perspective, relevant

information on ecosystem state has been obtained by identifying and analysing parasite communities (Marcogliese 2005, Vidal-Martinez *et al.,* 2010, Holmes 2014, Sures *et al.,* 2017).

Beyond the health of their individual hosts, parasite communities can reveal ecosystem stress, changes in host populations, food web structure and ecosystem functioning in response to ecological disturbances (Marcogliese 2001; 2005). In terrestrial ecosystems, Hudson *et al.,* (2006) suggested that a "healthy" ecosystem is one that contains a richness and an abundance of parasite taxa (Marcogliese, 2005). This contradicts past ideas of "stressed" ecosystems or hosts where negative interactions like parasitism were expected to increase in line with decreasing physiological health (Odum, 1985).

The ability of parasite taxa to reflect ecosystem state is varied, and is likely context and taxa dependent (Khan & Thulin, 1991, Sures *et al.,* 2017). Thus, when selecting potential parasites to assess MEH or environmental state, detailed knowledge of life histories, phylogenetic lineages, response to different stressors and the host's immune system is needed (Vidal-Martínez, 2010). The biology and life histories of parasite taxa affect their suitability as indicators and sensitivity to environmental stressors (Marcogliese, 2005, Blanar *et al.,* 2009). In particular, parasites with free-living stages and that require multiple hosts to complete their lifecycle are sensitive to ecosystem disturbance (Sures *et al.,* 2017, references therein).

Trematodes, i.e.flukes, are a class within the phylum Platyhelminthes (flatworms) contains the class Trematoda that subdivides into subclasses Aspidogastrea and Digenea. Digenea is the larger subclass; it contains 77 families, 18,000 species and has high rates of species descriptions still occurring (Poulin 2014, Costello 2016). It

is one of the most successful groups of parasitic trematodes (Cribb *et al.,* 2003, Olson *et al.,* 2003). Digenean trematodes are predominately endoparasites of vertebrates with complex lifecycles that usually require two invertebrate intermediate hosts and a final vertebrate host (heteroxenous) (Fig. 1). Eggs are produced through sexual reproduction of adult worms in the vertebrate host. Eggs are released with host faeces and hatch into free living ciliated infective stages (miracidia) either in the water column or after consumption by the first intermediate host (Sousa, 1994, Esch *et al.,* 2001). In the first intermediate host, usually a mollusc, miracidia reach a species-specific tissue or site of infection and transform into asexual reproductive bodies (sporocysts). Sporocysts form additional sporocysts or another larval stage with a mouth and primitive gut (rediae). Rediae can ingest host tissue and other larval stages of trematode species (Kuris 1990, Esch *et al.,* 2001). Within sporocysts and rediae, another free living infective stage (cercariae) develop. Cercariae, once mature, are released into the water column. Cercariae are usually short lived, do not feed, and contain limited reserves of energy (Thieltges & Rick, 2006, Haas *et al.,* 2008). In the water column, cercariae can actively seek out second intermediate hosts or suitable substrates to encyst within or on (metacercariae) (Haas 2003, Haas *et al.,* 2008). Ingestion of metacercariae by the final vertebrate host completes the lifecycle of the trematode (Esch *et al.,* 2001, Haas *et al.,* 2008).

Larval digenean trematodes are capable of infecting a compatible molluscan host by evading the host's internal defense system through a variety of approaches. The trematode larvae can inhibit a host's immune response through the downregulation of hemocyte activity, the release of secretory-excretory products which block or destroy hemocyte receptors or mask or mimic molecular signals that promote hemocyte defense responses (Adema & Loker, 1997).

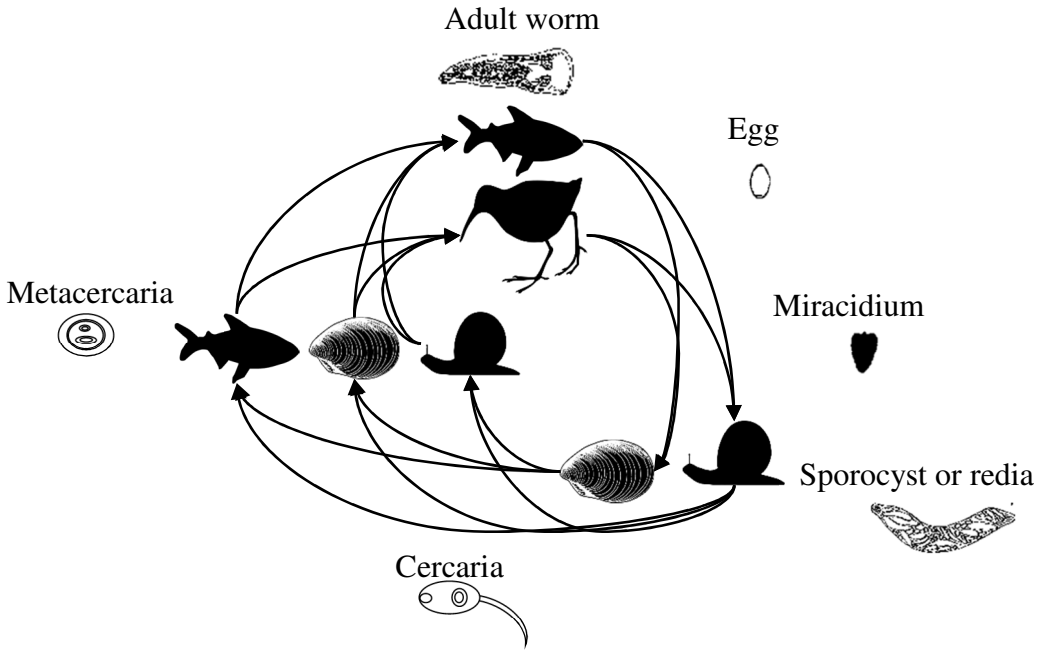

Fig. 1. Schematic of generalised 3-host lifecycle of marine digenean trematodes. Adult worms live in the final host, generally a vertebrate. Eggs are shed into the water column with host faeces. The eggs then hatch into ciliated organisms, miracidia, in the water column or within the second intermediate host, usually a mollusc. In the first intermediate host, miracidia develop into sporocysts which asexually produce rediae or more sporocysts. Large numbers of free living infective stages, cercariae are released from sporocysts or rediae and encyst in second intermediate hosts or on substrate. Upon consumption by a final host these cysts, metacercariae, transform into adult worms. Please note schematic is not to scale.

Trematodes as indicators of MEH

Parasitic trematodes, in particular have been suggested as suitable indicators of "ecosystem health" (Marcogliese 2005, Sures *et al.,* 2017). Since trematodes require multiple hosts to complete their lifecycles, any perturbance to their transmission or hosts will likely result in a decline in trematode abundance. Trematode communities are suggested to inform on the health of the host organism, its trophic position within the food web and the presence in the ecosystem of any other organisms that are involved in their life cycles (Marcogliese 2005b). Trematodes that utilise invertebrates as primary intermediate hosts, can alter host shell growth, zonation on the shore, behaviour, attachment strength and the fecundity of their gastropod first intermediate hosts (Davies & Knowles, 2001, McCarthy *et al.,* 2004, O'Dwyer *et al.,* 2014a; 2014b). Despite the higher occurrence of metacercariae relative to sporocysts, the effects of trematodes on second intermediate hosts are less well studied (Sousa, 1991, Mouritsen & Poulin 2002). Trematodes that utilise bivalves as second intermediate hosts can affect the host's size, respiration rate and movement (Mouritsen & Poulin, 2002a, Stier *et al.,* 2015). Laboratory studies suggest that heavy infections of metacercariae can negatively affect an invertebrate hosts' survival, movement and respiration rate (Stier *et al.,* 2015).

Meta-analyses carried out by Lafferty (1997), Blanar *et al.,* (2009) and Vidal-Martinez *et al.,* (2010) found that trematodes were consistently and negatively affected by a wide range of pollutants: pesticides, polycyclic aromatic hydrocarbons (PAHs), heavy metals, sewage, pulp and paper waste. However, the response of trematodes to eutrophication differed between reviews (Lafferty, 1997, Blanar *et al.,* 2009, Vidal-Martinez *et al.,* 2010). In reviews conducted by Lafferty (1997) and Vidal-Martinez *et al.,* (2010) eutrophication encouraged trematode infection, while Blanar *et al.,*

(2009) found the reverse. Furthermore, larval free-living stages of trematodes, cercariae, are sensitive to environmental conditions (Pietrock & Marcogliese, 2003). Trematodes have been proposed as indicators of biodiversity as the abundance of larval stages have previously been found to predict the abundance of their final reptilian (Byers *et al.,* 2011) and avian hosts (Huspeni & Lafferty, 2004, Hechinger and Lafferty, 2005, Shea *et al.,*2012).

Larval trematode communities are also affected by host habitat quality (Cross *et al.,* 2001; 2005, Marcogliese 2005a, Palm *et al.,* 2009). Poor habitat quality is suggested to negatively affect trematodes by impairing the infection success of their free living infective stages (Cross *et al.,* 2001; 2005). *Littorina littorea* when acting as a first intermediate host of trematodes in a polluted environment produced less viable cercariae (Cross *et al.,* 2001; 2005). Trematode cercariae in hosts in poor quality habitats, accumulated heavy metal pollutants and had impaired swimming ability and longevity (Cross *et al.,* 2001; 2003; 2005). Trematode communities in disturbed or impacted habitats require time to recover to pre-impact levels (Huspeni & Lafferty, 2004, Morley & Lewis, 2006, Aguirre-Macedo *et al.,* 2011). Trematode communities of gastropods *Lymnaea stagnalis* (Linnaeus, 1758) significantly declined during and after the restoration of a canal in the United Kingdom (Morley & Lewis, 2006). In a restored Californian salt marsh, trematode communities of the Californian horn snail, *Cerithideopsis californica* (Haldeman, 1840), in impacted sites took 6 years to reach abundances and richness recorded in reference sites (Huspeni & Lafferty, 2004). Similarly, it took several years after a hurricane, for trematode communities of the horn snail *Cerithideopsis pliculosa* (Menke, 1829), in the Yucatán Peninsula, Mexico to reach pre-impact abundances (Aguirre-Macedo *et al.,* 2011). In Northeast American salt marshes, anthropogenic factors: road density, nitrogen and metal contaminants, had a greater effect on the abundance of trematode communities of

Tritia obsoleta (Say, 1822) than the abundance of parasites' final hosts (Altman & Byers, 2014). Altman & Byers, (2014) speculated that these factors affected trematode communities via the abundance of their final fish hosts.

Trematodes commonly harboured within mullet species *Liza aurata* (Risso, 1810) and *Liza ramada* (Risso, 1827), were absent from a heavily polluted Mediterranean estuary (Dzikowski *et al.,* 2003). Previous work on the variation of trematode communities in relation to ecological quality has focused on fish (Williams & Mackenzie, 2003, Marcogliese, 2005, Sures *et al.,* 2017). However, utilising fish as hosts in parasitological surveys can be problematic due to their large spatial ranges, limited availability for particular species and the ethical dilemmas of utilising destructive techniques on vertebrates (Khan & Thulin, 1991, Jackson *et al.,* 2001, Costello *et al.,* 2016). Low mobility and restricted diets of hosts are factors that may generate stability and predictability in their parasite communities by contributing to regular and stable recruitment rates by different parasite species (Gonzalez and Poulin, 2005). Thus, in this thesis, the invertebrate-trematode relationship was considered the most appropriate host-parasite model to evaluate ecological quality and the role of surrounding ecological communities.

Trematode infection can have ecosystem-level effects, the extent of which is likely dependent on the abundance of the trematode, the induced alterations of the host and the host population size (Wood *et al.,* 2007, Clausen *et al.,* 2008, Mouritsen & Poulin, 2010). In the North Sea, the common periwinkle, *Littorina littorea*, when utilised as a primary intermediate host by trematodes, consumed significantly less algae relative to uninfected specimens. Thus, the structure of macroalgal communities in microcosm experiments was found to significantly differ between populations of trematode-infected and uninfected *L. littorea* (Clausen *et al.,* 2008). Trematode-induced changes

in intermediate hosts have implications for host population ecology, community interactions, nutrient flow between habitats and food web structure (Lefevre *et al.,* 2009). For example, in Californian estuaries, the killifish *Fundulus parvipinnis*, Girard 1854, infected with metacercariae were 10-30 times more likely to be consumed by avian predators (Lafferty, 2008). Behavioural changes in the New Zealand cockle *Austrovenus stutchburyi* (Wood, 1828) as a trematode second intermediate host resulted in decreased sediment disturbance, increased surface structural complexity and increased larval trematode abundance (Mourisen & Poulin, 2010).

Present knowledge of trematodes of intertidal biota in Irish coastal waters

On a global scale, parasitological studies on charismatic or commercial vertebrate species greatly outnumber those on invertebrate species and generally focus on a single host/parasite species (de Montudouin *et al.,* 2000, Thieltges *et al.,* 2006, Bordes & Morand 2009). Studies on marine microparasites outnumber those on marine macroparasites species (Orias *et al.,* 1978, Byrne *et al.,* 1999, O'Connell & Fives 2004, Lynch *et al.,* 2014). Work in an Irish context has had a similar focus, but has utilised a wide array of techniques, histological, genomic and macroscopic, to detect macro- and microparasites e.g. Fermer *et al.,* 2011a; 2011b, Callaway *et al.,* 2012, Lynch *et al.,* 2012; 2014, Firth *et al.,* 2017. In particular, the microparasites of commercial bivalves the Pacific oyster *Magallana gigas* (Thunberg, 1793), mussel *Mytilus* spp. (Linnaeus, 1758) and European flat oyster *Ostrea edulis* (Linnaeus, 1758) have received much attention in the literature e.g. Culloty *et al.,* 1999, Lynch *et al.,* 2010, Lynch *et al.,* 2012.

On the southwest coast of Ireland, intertidal bivalves were the biota most commonly screened for macroparasites (Fermer *et al.,* 2011, Lynch *et al.,* 2012, Morgan *et al.,* 2012) and gastropods (Prinz*et al., et al.,* 2010). In these studies, trematodes were found to be a major component of macroparasite communities of common invertebrates on rocky shores (Prinz *et al.,* 2010) and of the bivalve, the edible cockle *Cerastoderma edule* (Linnaeus, 1758) in soft sediment shores (Fermer *et al.,* 2011a; 2011b). In the north of Ireland, trematodes have been recorded in several intertidal gastropods (Copeland *et al.,* 1987, Field & Irwin 1999, McCarthy *et al.,* 1999; 2000; 2002, Cross *et al.,* 2005), acorn barnacles *Semibalanus balanoides* (Linnaeus, 1767) (Irwin & Irwin 1980) and herring gulls *Larus argentatus*, Pontoppidan, 1763, (Irwin & Prentice 1976). Firth *et al.,* (2017) screened the common limpet *Patella vulgata* (Linnaeus, 1758) from rocky shores on the east and west coast of Ireland. Larval trematodes were abundant in *P. vulgata* for the majority of sites sampled.

OUTLINE

The present study focused on gaining more knowledge of the macroparasites of common marine invertebrate species in Irish coastal waters and relating them to the current known environmental parameters associated with the sites. The study aimed to evaluate if parasite communities, with a focus on trematodes, of biota screened varied in response to ecosystem quality as defined by policy directive classifications (Chapter 2 & 4), levels of statutory protection (Chapter 2, 3 & 5) or nutrient enrichment (Chapter 4).

Chapter 2 describes a year-long survey of the parasite communities of the common periwinkle *Littorina littorea*, common limpet, *Patella vulgata,* and dogwhelk *Nucella lapillus.* Gastropods were sampled from three rocky shores of differing Water Framework Directive classifications as well as from a marine reserve, Lough Hyne, on the Southwest of Ireland. It was hypothesised that shores of higher status would contain richer and more abundant parasite communities.

In **Chapter 3**, parasite communities of the common limpet *Patella vulgata* were surveyed within and outside the marine reserve Lough Hyne. The study aimed to investigate the potential of the marine reserve to act a reservoir of marine parasites due to its sheltered coastline and statutory protection. It was hypothesised that a higher occurrence and diversity of parasites would be observed within the boundaries of the marine reserve.

Chapter 4 examines parasite communities occurring in the European green shore crab, *Carcinus maenas*, and Ragworm, *Hediste diversicolor*, in three different estuaries of different nutrient status classifications according the Water Framework

Directive and characterised by physio-chemical parameters. Avian communities were surveyed at each estuary to estimate the number of potential role of final hosts of trematode taxa recorded.

In **Chapter 5**, the parasitological communities of subtidal blue mussels *Mytilus* spp. within and outside of Lough Hyne marine reserve are described. Also, the susceptibility of *Mytilus* spp., to the cercariae of the Digenean trematode *Echinostephilla patellae* was investigated following previous work identifying the bivalve as an intermediate host. Host stress was considered in terms of differing *Mytilus* spp. habitats, exposure to the dinoflagellate *Karenia mikimotoi* and simulated nutrient enrichment.

Chapter 6 provides conclusions of the study and reviews the results of preceding chapters, evaluates their significance and suggests future work.

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CHAPTER 2: Factors affecting parasite infection of three common intertidal gastropods on rocky shores of differing water quality on the coast of Ireland

ABSTRACT

Impacts of anthropogenic activities on marine ecosystems are addressed by legislation to protect and monitor coastal waters. Sentinel organisms have been widely used to indicate anthropogenic disturbance on rocky shores. Parasites on rocky shores are a common, but understudied, occurrence. Parasitic trematodes are a large component of macroparasite communities of intertidal fauna and have been proposed as indicators of ecosystem quality. This study investigated the variation in the abundance and richness of parasites, with a focus on trematodes, of intertidal invertebrates in relation to differing Water Framework Directive classifications. For a year, intertidal gastropods, the common periwinkle *Littorina littorea*, dog whelk *Nucella lapillus*, and common limpet *Patella vulgata* were sampled from three rocky shores with differing classifications. *Littorina littorea* and *P. vulgata* were sampled from a site of high water quality Lough Hyne Marine Reserve. Sampling of *L. littorea* and *P. vulgata* was restricted to bimonthly events as stipulated in a National Parks and Wildlife licence to minimise potential detrimental effects. Intertidal biota surrounding three host species were recorded, to evaluate potential disrupters of trematode transmission in the field. In total, 3,973 gastropods were screened and parasite taxa from four phyla were recorded: Annelida, Arthropoda, Ciliophora and Platyhelminthes. Nine trematode taxa from seven families were recorded. Parasite taxa were common and varied greatly over the temporal scales, host species and sites considered. There was no clear pattern in parasite taxa richness between sites related to WFD quality classification. Ciliates and cestodes were less common in the site of high quality. On shores of moderate quality, the highest and lowest number of trematode taxa, the highest and lowest prevalence of primary infections of *L. littorea* and the lowest prevalence of trematode metacercariae in *L. littorea* and *P. vulgata* were recorded. Water quality alone was not

considered a determining factor for richness, prevalence or intensities of parasites recorded.

2.1. INTRODUCTION

Parasites are an understudied but ubiquitous component of ecosystems (Marcogliese 2005, Poulin 1999; 2014, Poulin *et al.,* 2016), estimated to constitute 40% of all known species and form up to 75% of key links in food webs (Dobson *et al.,* 2008, Lafferty *et al.,* 2008). Parasites in contribute biodiversity and productivity to aquatic ecosystems (Poulin, 1999, Mouritsen & Poulin, 2002a). Parasite abundance and dynamics are affected by anthropogenic impacts (Lafferty 1997, Sures, 2008, Sures *et al.,* 2017). Meta-analyses revealed an association between environmental impact and parasite abundance (Lafferty 1997, Sures, 2008, Sures *et al.,* 2017). The magnitude of the association was attributed to the wide range of anthropogenic stressors, as well as to the inherent variability of parasite responses (Lafferty 1997).

Rich or abundant parasite communities are proposed to be indicative of the health or good status of an ecosystem rather than its decline (Marcogliese 2005, Hudson *et al.,* 2006). A heavily impacted or frequently disturbed ecosystem may inhibit the transmission of parasites with free living stages (Lafferty 1997, Sures, 2008, Sures *et al.,* 2017). Trematodes require multiple hosts to complete their life cycle and contain free living stages sensitive to a range of environmental stressors (Lafferty 1997, Pietrock & Mouritsen, 2003, Sures *et al.,* 2017). In the Yucatán, Peninsula, Mexico trematode communities of gastropods took several years to reach pre-impact levels following a hurricane in the area (Aguirre-Macedo *et al.,* 2011). The restoration of a Californian salt marsh, resulted in significant increases in the abundance of trematode communities of the California horn snail, *Cerithideopsis californica*. After six years, the occurrence of trematodes was equivalent to reference zones (Huspeni & Lafferty 2005). This recovery period is likely due to the time required to successfully transmit to multiple hosts.

Abundance of trematodes in invertebrates has been associated with increased water quality. In Ireland, water quality was not recorded to effect the diversity and abundance of parasites harboured by *Mytilus* spp. screened (Lynch *et al.,* 2014). Cercariae of *Cryptocotyle lingua* (Creplin, 1825) in *Littorina littorea* in poor quality water, due to high concentrations of heavy metal pollutants, were observed to have impaired swimming and longevity. This effect of environmental stress would have implications for their ability to find and infect the following host (Cross *et al.,* 2001a; 2003; 2005). In the Mondego Estuary, Portugal an increase in water quality, due to decreased nutrient loading led to heavy infection of the isopod *Cyathura carinata* (Kröyer, 1847) by trematode metacercariae (Ferreira *et al.,* 2007).

In Europe, two key directives for assessing coastal waters are the Water Framework Directive (WFD, 2006/60/EC) and the European Marine Strategy Framework Directive (MSFD, 2008/56/EC). Both the WFD and the MSFD require the development of cost effective monitoring tools to assess ecological status (ES) of European marine waters. In Ireland ES are assessed by the Environmental Protection Agency (EPA) and assigned to one of the five ES categories set out under the WFD, (high, good, moderate, poor and bad). Coastal bodies are assigned to quality classes by the EPA according to biological quality elements, physico-chemical parameters, environmental quality standards and hydromorphological quality elements (Clabby *et al.,* 2008, O'Boyle *et al.,* 2010, Bradley *et al.,* 2015).

The transmission of trematodes could be affected by the (not mutually exclusive) mechanisms of predation, physical disturbance, hyperparasitism, toxic exudates and the infection of unsuitable hosts (Thieltges *et al.,* 2008). Surrounding biota can act as an alternative host or a "decoy" to free living trematode stages. The decoy mechanism has been found in the field in the Wadden Sea with a number of intertidal bivalves. Mouritsen *et al.,* (2003) and Thieltges and Reise (2007) found that cockles *Austrovenus stutchburyi* and *Cerastoderma edule* (Linnaeus, 1758) in close proximity or surrounded by rings of *Mytilus* spp., harboured fewer metacercariae due to the increased infection of the nearby mussels. Laboratory studies have shown that a range

of rocky shore biota can physically disturb and consume trematode cercariae, which impairs the trematode's ability to reach or infect to their second intermediate hosts (Thieltges *et al.,* 2008, Prinz *et al.,* 2009, Studer *et al.,* 2013, Welsh *et al.,* 2014).

Intertidal gastropods have been used in biomonitoring programmes, are key host species for trematodes and influential consumers in the rocky intertidal (Werding 1969, Thompson *et al.,* 2002, Prinz *et al.,* 2010a, O'Connor *et al.,* 2011, Atalah and Crowe, 2012). *Littorina littorea*, *Patella vulgata* and *Nucella lapillus* were chosen as the hosts for this study due to their Atlantic-wide distributions, use in biomonitoring programmes and differing ecological roles of an omnivore grazer, macro-grazer and predator, respectively (Moore 1937, Crothers 1985, Crowe *et al.,* 2000, Jenkins *et al.,* 2001b, Atalah & Crowe, 2010). The aims of the research were to examine the effect of (a) site related water quality classifications according to WFD on parasite communities and (b) investigate the effect of surrounding biotic communities. To account for seasonal variation and host effects, the study was conducted for a year and parasitism in gastropod populations was recorded monthly or bimonthly in some sites.

2.2 MATERIALS AND METHODS

2.2.1 Study Sites

In April 2014, preliminary surveys of seven rocky shores of differing WFD ecological classifications were carried out. The intertidal zone of Cusheen (51°31'47.65"N, 9°31'36.30"W), Cuskinny bay (51°51'26.9"N 8°15'59.0"W), Lough Hyne (51°30'1.55"N, 9°18'22.06"W), Sandycove (51°40'34.20"N, 8°31'24.30"W), Tranabo (51°29'52.7"N 9°17'07"W), Whitegate (51°50'18.11"N, 8°13'45.72"W) and Whiddy Island (51°40'53.5"N 9°30'20.9"W) were surveyed with the methodology detailed in Section 2.2.2 page 40. Shores were chosen to allow comparison between different WFD classifications and sufficient host species populations to support year-long monthly sampling. At the time of surveying only classifications based on data from 2007-2009 were available. No coastal water body was classified as of "poor" or "bad" quality by the Environmental Protection Agency, (EPA) (O'Boyle *et al.,* 2010). From the seven shores, two "moderate" shores, one "good" and one marine reserve of "high" quality were chosen to sample.

Cusheen was a shore of "moderate" quality status. The rocky shoreline had a gentle slope that graduated into sand and mud in the subtidal zone. The sheltered south-west facing rocky shore is situated in Roaringwater bay within a Special Area of Conservation (SAC) (O'Boyle *et al.,* 2010). Lough Hyne is a marine reserve, covers an area of 0.8 km^2 and is an extremely sheltered shore with restricted tidal exchange with outer waters, due to the dimensions of the connecting channel. Lough Hyne was not evaluated by the EPA but is considered to have high status water quality (Jessopp *et al.,* 2010, Lynch *et al.,* 2014). Sandycove is a semi-exposed south facing shore in Kinsale and is considered to be of "good" quality status by the EPA. Whitegate is a west facing bay on the east side of Cork harbour and is classified as "moderate" quality by the EPA. Whitegate bay is within the boundaries of a Special Protection Area (SPA), contains Ireland's only oil refinery which experienced an oil spill in 1997 (EPA, 1997).

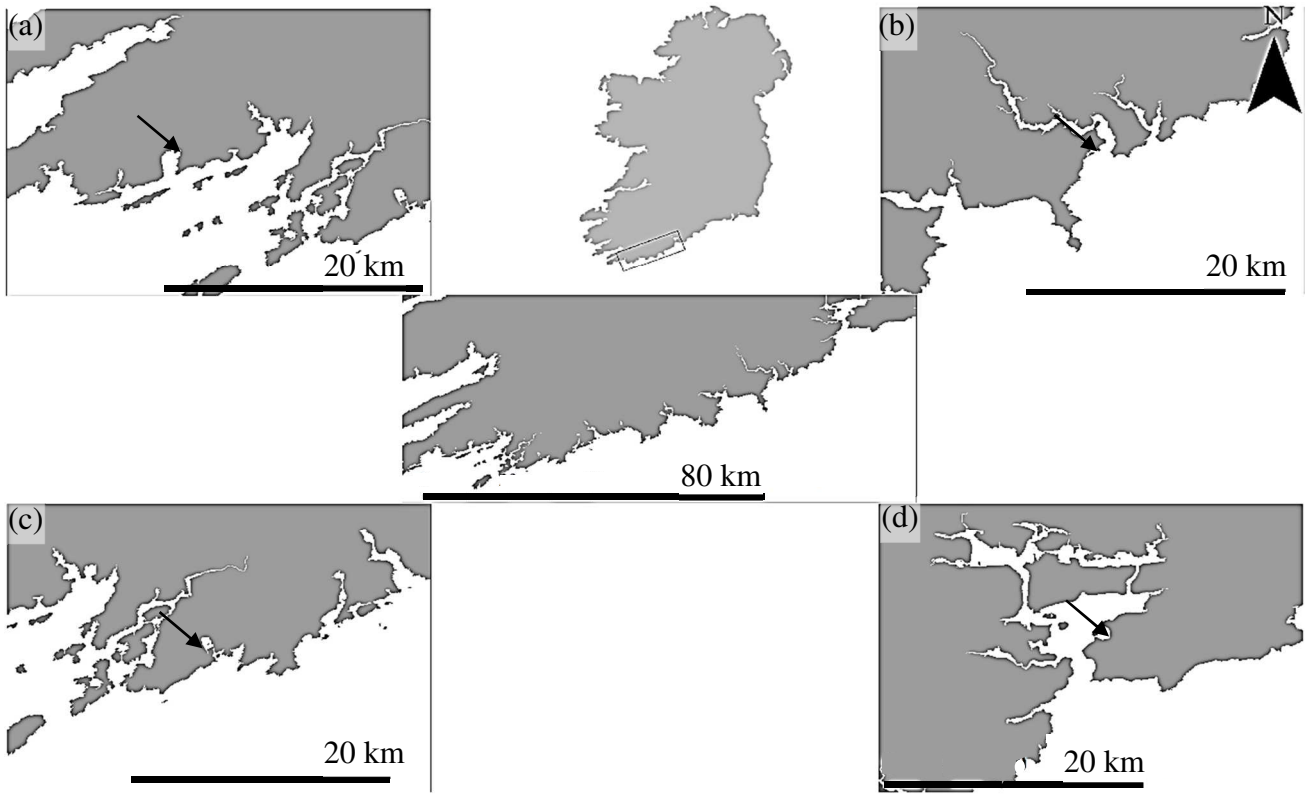

Fig. 1 Map of study sites on the southwest of Ireland (a) Cusheen, (b) Sandycove, (c) Lough Hyne & (d) Whitegate. Adapted from Environmental Protection Agency Water Quality shapefile© Ordnance Survey Ireland and Government of Ireland protected by the Copyright Act 1963, Copyright (Amendment) Act 1987, Copyright and Related Rights Act 2000 and EU Copyright and Database Directives.

2.2.2 Field collections

To account for temporal variation a minimum of 30 each of *L. littorea, N. lapillus* and *P. vulgata* were sampled monthly from Cusheen, Sandycove and Whitegate from May 2014 to April 2015. From May 2014 to March 2015 30 each of *L. littorea* and *P. vulgata* were sampled every second month from Lough Hyne as permitted by the National Parks and Wildlife Service. Host densities were calculated per m^2 from counts within randomised 0.25 m^2 quadrats on the low shore. Low shore was sampled to allow comparison e with Lough Hyne's small tidal range, build on the work of Prinz *et al.,* (2010b) and facilitate the detection of trematodes. Gastropods with shell heights greater than 10 mm were sampled from exposed rock at low shore (Byers *et al.,* 2015). Smaller and sexually immature specimens are less likely be to harbour parasite taxa (Moore, 1937, Lauckner, 1980; 1983). Low shore of the intertidal was considered to the zone between the mean tidal level (MTL) and to mean of the low water spring tides (MLWS). To minimise the variation in microhabitat of gastropods, rock pools were excluded from sampling. Intertidal biota known to affect the transmission of trematodes were recorded, (see Results Table 3) (Thieltges *et al.,* 2008, Prinz *et al.,* 2009, Studer *et al.,* 2013, Welsh *et al.,* 2014). Barnacle species were classified on the SACFOR abundance scale according to Crisp & Southward (1958) and modified by Hiscock (1981) with S representing super abundant, A: abundant, C: common, F: frequent, O: occasional, and R: rare (SACFOR). The presence of polychaetes, decapod crabs, anemones and non-host gastropods was noted. Algal species were recorded as percentage cover in 0.25 m^2 quadrats.

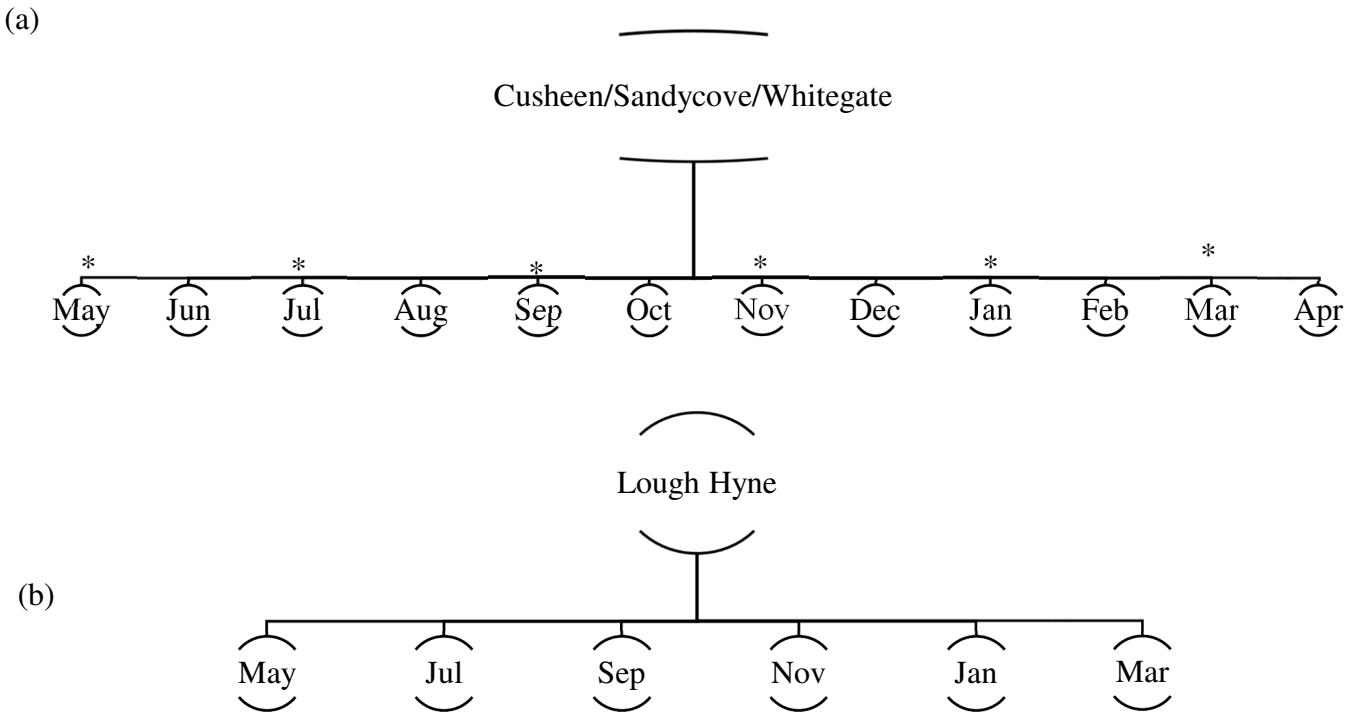

Fig. 2 Schematic diagram of sampling programme for (a) the monthly collection of *Littorina littorea, Nucella lapillus* and *Patella vulgata* and (b) bimonthly sampling of *N. lapillus* and *P. vulgata* from marine reserve Lough Hyne. Asterisks denote the subsampled data from Cusheen, Sandycove and Whitegate for comparison with Lough Hyne

2.2.3 Processing

In the laboratory encrusting biota were removed from shells and discarded. This was done to avoid interference of encrusting biota with gastropod trematodes (Prinz *et al.,* 2009, Welsh *et al.,* 2014) and to avoid including parasites of encrusting biota in counts. Gastropods were weighed to the nearest 0.01 g on an electronic balance (OHAUS Adventurer® Pro balance model AV3102). Prior to dissection, shell height, length, and width were measured to the nearest 0.05 mm with Vernier callipers (Draper® 4817P) (see Fig. 2). Shells were cracked or removed from gastropods by first severing the abductor muscle. A second incision was then made to sever the cerebral ganglion, then gastropods were sexed: *L. littorea* according to Graham (1969), Gibbs *et al.,* (1988) and Reid *et al.,* (1996), *N. lapillus* according to Bettin *et al.,* (1996), and *P. vulgata* according to Orton (1919) and Blackmore (1969). *Patella vulgata* is a sequential hermaphrodite where male gonads develop prior to changing to female gonads (protandry). *Patella vulgata* was classified as female with the presence of greenish eggs, male with orange sperm, or hermaphroditic with both eggs and sperm. If neither were present, limpets were classified as undifferentiated (Orton, 1919, Blackmore, 1969). The visceral mass and foot tissues of *P. vulgata* were separated prior to compression. Whole live mounts were compressed between ¼ inch thick glass slides (Kompressorium 23 x 5 cm Hauptner-Herberholz®) and examined using transmitted light with a stereomicroscope (Nikon SMZ645) and compound microscope (Nikon Alphaphot-2 YS2-H) at magnifications of x8 and x40 respectively. Parasitological terminology followed that of Bush *et al.,* (1997). Prevalence (*P*) was defined as the percentage of infected hosts and intensity (*Ins*) was defined as the average number of parasites within infected hosts. Helminth parasites were counted individually, whereas only the presence or absence of ciliates, cestodes, copepods, mites, nematodes, polychaetes parasite taxa were recorded. This was due to the inability to distinguish individuals for counts. Images were taken with the compound microscope Nikon ECLIPSE 80i and NIS-Elements Microscope Imaging Software.

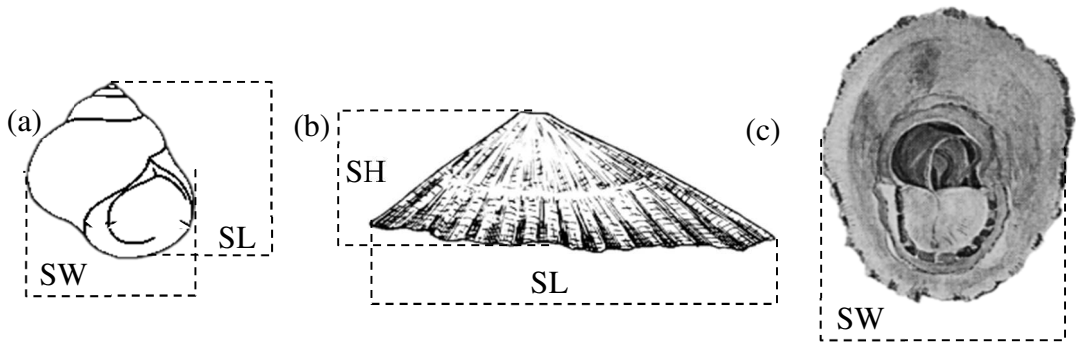

Fig. 3 Illustrations of morphometric measurements taken from (a) *Littorina littorea* and *Nucella lapillus*, modified from Reid (1996), and (b) & (c) *Patella vulgata* modified from Orton (1919). SH represents Shell height, SL Shell length and SW Shell width.

2.2.4 Parasite identification

Parasite taxa were identified by morphology according to Nicoll (1907), Stunkard (1950), Crewe (1951), James (1968a), Werding (1969), Lauckner (1980), MacKinnon & Burt (1983), Kollien (1996) and Buckland-Nicks *et al.,* (2013). Cestode taxa were identified by the lack of an alimentary canal and the presence of the protoscolex (precursor to attachment sucker) and cercomer (caudal appendage). Larval trematodes were distinguished according to their size and if present; acetabula (suckers), colouration, excretory systems, tail, stylet and eyespots. For metacercariae the cyst wall or collar spines, again if present, were also used to distinguish between taxa. As has been done in previous studies *Cercaria parvicaudata* (Stunkard and Shaw, 1931) and *Renicola roscovita* (Stunkard, 1932) primary intermediate infections were grouped as *Renicola* spp. due to the subjectivity of differentiating between the colours yellow and orange. *Himasthla* and *Gymnophallus* species were also grouped in their respective genera. Trematode identification was confirmed to the family level by Drs David Thieltges and Xavier de Montaudouin (pers. comm.).

2.2.5 Statistical Analysis.

Analysis was carried out in R studio version 1.0.136 (R Core Team 2015). Parasitology terms follows Bush *et al.,* (1997). Statistical significance was considered with $P<0.05$. When comparing with the marine reserve, Lough Hyne, a subset of data from the three shores was used (Fig.3). Normality of distributions was assessed first visually and then formally by the Shapiro–Wilk test. Normality of distributions was assessed first visually and then formally by the Shapiro–Wilk test. Homoscedasticity was determined using a Levene's Test for Equality of Variances. Prior to analysis, non-normal data were transformed; count data were transformed via square root, proportional data transformed via arcsine and continuous data were transformed via log. If transformation failed to normalise the distribution of data, non-parametric tests were carried out.

2.2.5.1 Summary statistics

Normally distributed data was summarised with an arithmetic mean and standard deviation. For parasite prevalence, non-normally distributed data, 95% Clopper Pearson Confidence intervals have been found to be conservative and appropriate measure of variation (Reiczigel 2003, Reiczigel *et al.,* 2008). 95% Clopper Pearson Confidence were calculated with the r package "PropCIs" where presence/absence of parasite taxa were considered to be success/fail of a Bernoulli experiment. Parasite intensity typically has highly aggregated (right-skewed) frequency distribution and so was summarised by a median and the range of infection (Rozsa *et al.,* 2000).

2.2.5.2 Effect of quality

Gastropod host densities and morphometric measurements were compared with the parametric one-way analysis of variance (ANOVA) and a post hoc Tukey Honest Significant Differences (Tukey HSD) test. (Underwood 1997). ANOVA and Tukey HSD were carried out with the package "car" and "stats", respectively.

Comparisons of parasite prevalences and intensities were done with nonparametric test a Pearson's chi square with Yates continuity correction and Kruskal-Wallis chi square, respectively. If expected values were too low (<5) for a Pearson's chi square, a Fisher's Exact test was used. Posthoc comparisons were made with the nonparametric Pairwise Wilcoxon Rank Sum Tests andc arried out with the r package "stats".

2.2.5.3 Effect of surrounding biota

Pearson Correlations were used to investigate relationships between parasite taxa their host morphometrics, abundance surrounding biota and co-occurring parasite taxa. The effects of month, site and size of gastropods on the composition of parasite communities were investigated using non-metric multi-dimensional scaling (nMDS) and non-parametric one-factorial or two-factorial PERMANOVA procedures based on Jaccard similarity indices of non-transformed prevalence or intensity data. The effect of sampling effort (number of quadrats or hosts) on the number of parasite species across all sites, separately for each gastropod species was evaluated by Pearson correlations. was used for statistical comparison with the marine reserve.

2.3. RESULTS

2.3.1 Host morphometrics

The smallest *L. littorea* sampled were from Lough Hyne (F=25.43, df=3, P<0.0001) (Table 1). Significantly larger specimens (F=84.62, df=2, P<0.0001) and higher densities (F=25.43, df=2, P<0.0001) of *L. littorea* were sampled from and recorded in Sandycove (Table 1). Larger *N. lapillus* specimens were sampled from Whitegate and Cusheen relative to Sandycove (F=10.19, df=2, P<0.0001), while significantly higher densities of *N. lapillus* were recorded in Sandycove relative to Whitegate (F=5.18, df=2, P<0.001). Larger *P. vulgata* were sampled from Whitegate relative to the other sites (F=13.42 df=2, P<0.0001). Significantly higher densities of *P. vulgata* were recorded in Sandycove relative to other sites (F=133.4, df=2, P<0.0001). The lowest density of *P. vulgata* recorded was in Lough Hyne (F=125.5, df=3, P<0.0001).

Table 1. The size and density ($\bar{x}$±SD) per m^2 of gastropod host screened at each location and for all months combined 2014/15.

Host	Site			
	Cusheen	Lough Hyne	Sandycove	Whitegate
Littorina littorea				
n	498	195	400	479
Shell height	18.32 (± 4.51)	17.11 (± 5.00)	24.39 (±5.70)	21.68 (± 10.10)
Density	42 (± 46)	19 (± 34)	17 (± 36)	16 (± 27)
Nucella lapillus				
n	273	NA	301	382
Shell height	28.47 (± 5.83)	NA	26.15 (±5.17)	28.51 (± 5.65)
Density	9 (± 10)	NA	10 (± 15)	13 (± 16)
Patella vulgata				
n	429	216	410	390
Shell height	13.47 (± 4.91)	12.05 (± 4.29)	12.14 (±5.78)	14.28 (± 6.14)
Density	58 (± 42)	41 (±32)	62 (± 54)	24 (± 23)

2.3.2 Parasite taxa

Of the host species screened, five macroparasite taxa were recorded; halacarid mites, the burrowing polychaete *Polydora ciliata* (Johnston, 1838), harpacticoid copepods, the cestode *Ophryocotyle insignis,* Loennberg, 1890, larval nematodes and nine genera of juvenile trematodes. One microparasite taxon was recorded; ciliates were recorded and were predominantly in *L. littorea* and *P. vulgata* (Table 1). Prevalence of parasite infection significantly differed between gastropod species (χ^2=1007.2, df=2, P<0.0001) (Fig 3 (a), (b)). *Patella vulgata* and *N. lapillus* had respectively the highest and lowest prevalence of infection (P<0.0001) (Fig.4). Co-infection between parasitic taxa was significantly more common in *P. vulgata* than in other gastropod hosts screened (χ^2=531.7, df=2, P<0.0001) (Fig. 4). Ciliates, most likely the species *Leiotrocha patellae* (Cúenot, 1891), and trematodes were the two most commonly cooccurring parasite taxa in *P. vulgata.* The prevalence of ciliates and trematodes in *P. vulgata* were positively correlated (r=0.09, P<0.05).

2.3.2.1 Non-Trematode parasite taxa

Ciliates were the second most common parasite taxa recorded, 16% of all gastropods screened harboured ciliates (Fig. 4). The polychaete *P. ciliata* occurred in all hosts at all sites and had an overall prevalence of 10% (Fig.4). Halacarid mites occurred in all host species. The cestode *O. insignis* solely occurred in *P. vulgata* and only in 3% of specimens screened. *Patella vulgata*, relative to the other host species, were significantly more infected by copepods (χ^2=79.91, df=2, P<0.001), ciliates (χ^2=189.81, df=2, P<0.0001), mites (χ^2=30.86, df=2, P<0.001), *P. ciliata* (χ^2=88.936, df=2, P<0.0001) and trematodes (χ^2=657.68, df=2, P<0.0001). Nematode prevalence was low (<1%) and did not differ between host species screened.

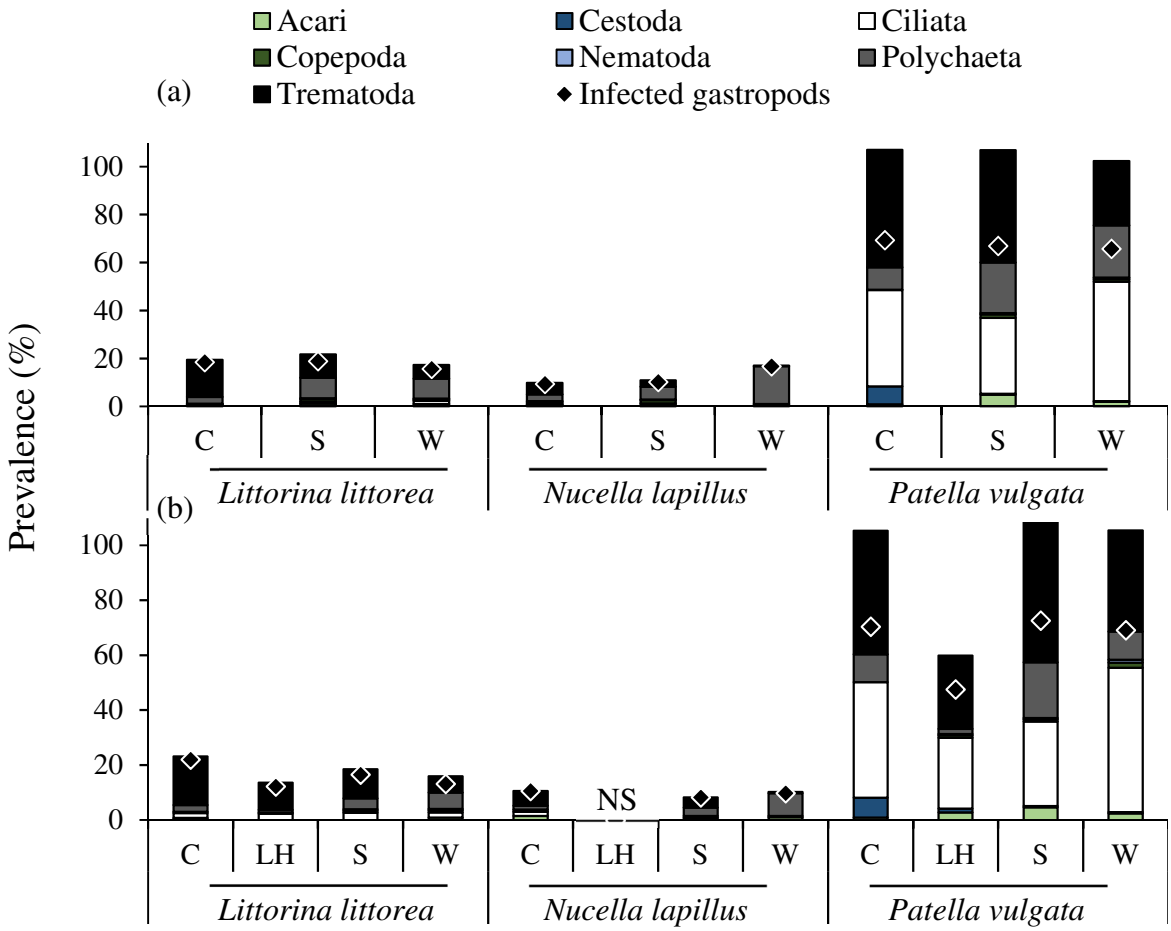

Fig. 4 (a) Overall prevalence of parasite taxa in the three gastropod hosts screened per site for all months combined. C represents Cusheen, S Sandycove and W Whitegate. (b) Overall prevalence of parasite taxa in three gastropod hosts from each site with bimonthly dataset for comparison with Lough Hyne Marine Reserve, LH. NS represents not sampled, as *Nucella lapillus* was not sampled from Lough Hyne due to insufficient population size.

2.3.2.2 Trematodes

Trematodes were the most common parasite recorded in the study for each host species (Fig. 4). The nine trematode taxa recorded were from seven families Echinostomatidae, Heterophyidae Renicolidae, Philophthaimidae, Notocotylidae, Opecoelidae, and Fellodistomidae. *Littorina littorea* had the richest trematode community of all hosts screened (Table 2). *Littorina littorea* were mainly second intermediate hosts for trematodes, with 7.69% infected with metacercariae and 3.65% with rediae or sporocysts. *Nucella lapillus* had the lowest richness and prevalence of trematode taxa with only 2.31% infected. *Patella vulgata* was the most commonly infected host and, overall, 41% harboured trematodes. *Echinostephilla patellae* (Fig. 6(b)) and *Renicola* spp. (Fig. 6(c)) metacercariae dominated trematode communities of *P. vulgata* and *L. littorea,* respectively (Table 2). Co-infection between two trematode species was rare and was found only in the form of *Renicola* spp. metacercariae and *Cryptocotyle lingua* sporocysts in *L. littorea.*

Parasite taxa in both *L. littorea* and *P. vulgata* varied between months sampled (Figs. 7&8). In all sites sampled, temporal variation in primary trematode infection in *L. littorea* was not statistically significant (Fig. 8). *Echinostephilla patellae* prevalence significantly differed between months for Cusheen (χ^2=54.82, df=11, P<0.0001), Lough Hyne (χ^2=21.90, df=5, P<0.001), Sandycove (χ^2=45.57, df=11, P<0.0001) and Whitegate (χ^2=73.23, df=11, P<0.0001).

Table 2. The prevalence and range (min-max) of intensity in brackets of trematode taxa recorded in gastropod species screened. Calculated for all sampling events in 2014/15 combined. NS denotes not-sampled as populations of *Nucella lapillus* in Lough Hyne were insufficient to allow yearlong sampling.

Host species	Trematode species	Cusheen	Lough Hyne	Sandycove	Whitegate	Total prevalence
SPOROCYST						
(Primary intermediate hosts)						
Littorina littorea	*Paramonostomum chabaudi*[*1](Van Strydonck, 1965)	0.00	0.00	0.50	0.00	0.20
	Cryptocotyle lingua	0.87	3.76	1.49	1.41	1.63
	Himasthla spp. (Dietz, 1909)	0.40	0.47	0.00	0.00	0.27
	Podocotyle atomon (Rudolphi, 1802)	0.22	0.47	1.24	3.74	1.56
	Renicola spp.	0.66	0.00	0.00	0.00	0.07
Nucella lapillus	*Parorchis acanthus* (Nicoll, 1906) Nicoll, 1907	0.00	NS	0.33	0.00	0.00
METACERCARIAE						
(Second intermediate hosts)						
L. littorea	*Renicola* spp. (Stunkard, 1932)	15.72 (1-875)	3.76 (1-5)	6.70 (1-133)	1.17 (1-8)	7.13
	Echinostephilla patellae	0.93 (1-8)	0.47 (1)	0.75 (1)	0.47 (1-6)	0.68
	Himasthla spp.	0.00	0.47(1)	0.00	0.00	0.06
N. lapillus	*Renicola* spp.	1.20 (1-2)	NS	3.96 (1-12)	0.21(1)	1.26
	E. patellae	1.12 (1-4)	NS	0.00	0.00	0.31
Patella vulgata	*E. patellae*	48.60 (1-88)	26.61 (1-35)	47.60 (1-131)	26.59 (1-44)	40.11
	Gymnophallus spp.[*2] (sensu, Crewe, 1951)	0.25 (103)	0.00	0.00	0.00	0.07
	Renicola spp.	0.79 (1)	0.00	0.00	0.28 (1)	0.28
ADULT WORM						
N. lapillus	*Proctoeces maculatus*[*3] (Looss, 1901)	0.44 (1-2)	NS	0.00	0.00	0.10
P. vulgata		0.23 (1)	0.00	0.00	0.00	0.07

[*1] formally *Cercaria lebouri* (Van Strydonck, 1965)
[*2] "Cercariae B"
[*3] formally *Proctoeces buccini* (Loos-Frank, 1969)

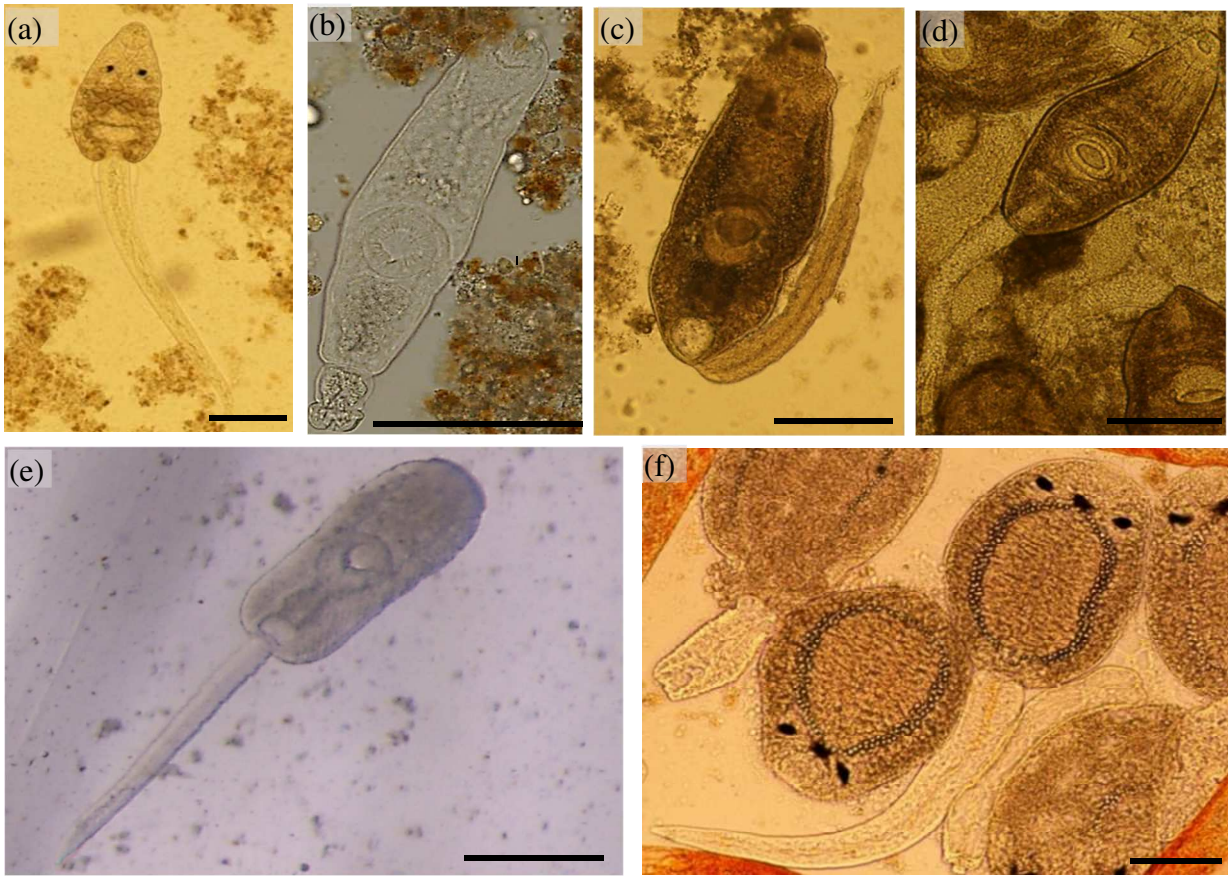

Fig. 5. Photomicrographs of trematode cercariae of the following taxa, scale cars in brackets (a) *Cryptocotyle lingua* (100µm) (b) *Podocotyle atomon* (100µm), (c) *Himasthla* spp. (100µm), (d) *Parorchis acanthus* (200µm), (e) *Renicola* spp. (100µm) and (f) *Paramonostomum chabaudi* (100µm)

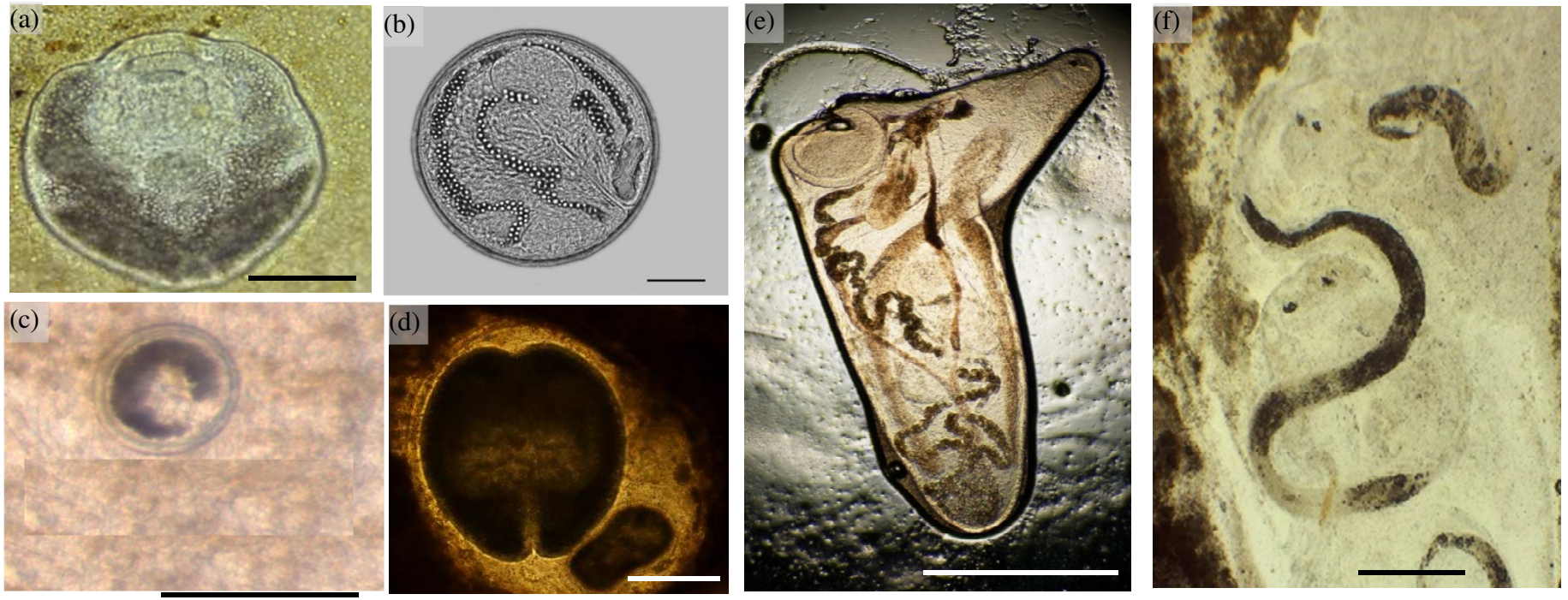

Fig. 6. Photomicrographs of helminth taxa recorded in the with study, scale bar measurements provided in brackets. (a) metacercariae of *Gymnophallus* spp. (50µm) reported as "Cercaria B" by Crewe (1951) (b) metacercariae of *Echinostephilla patellae* from Prinz *et al.,* (2009) (50µm) (c) metacercariae *Renicola* spp. (200µm) (d) cysticercoid of *Ophryocotyle insignis* (200µm) (e) Adult worm of *Proctoeces maculatus* Removed from *Nucella lapillus* (1000µm) (f) Juvenile stage of a nematode (200µm).

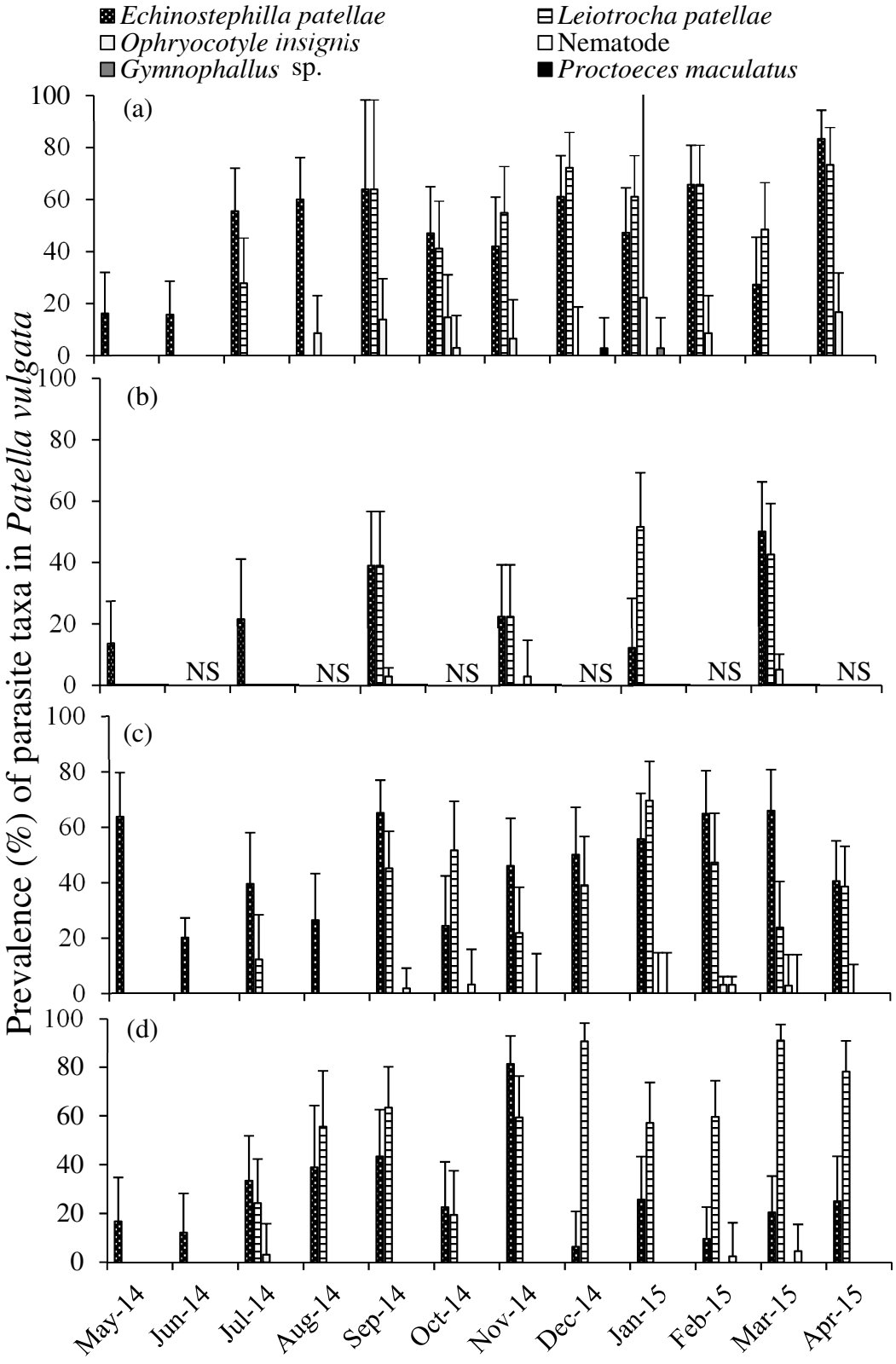

Fig. 7 Temporal variation in parasite taxa in *Patella vulgata* in (a) Cusheen, (b) Lough Hyne, (c) Sandycove and (d) Whitegate. NS represent not sampled as Lough Hyne was only sampled & surveyed every second month. Error bars are 95% Clopper Pearson Confidence Intervals.

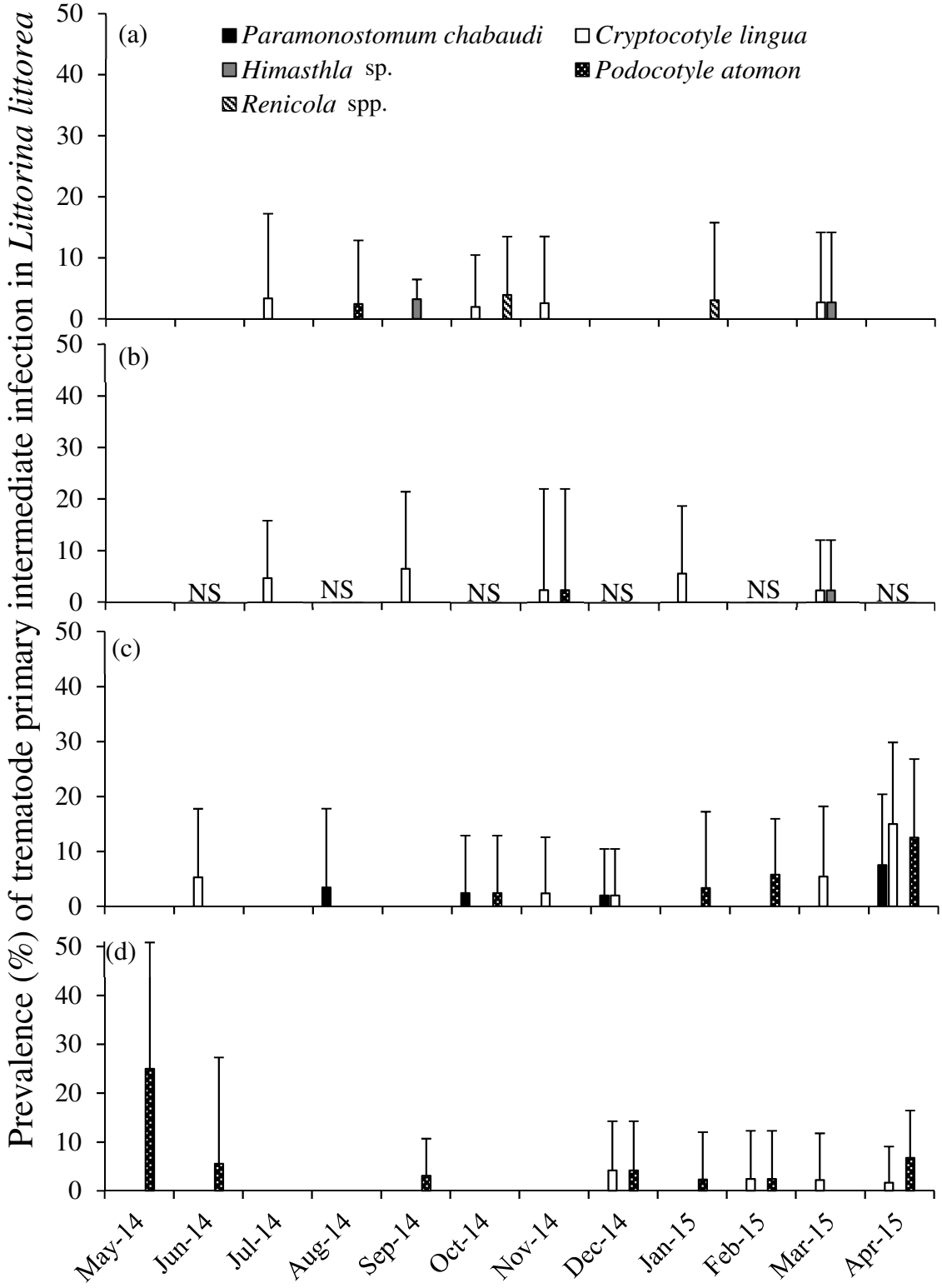

Fig. 8 Temporal variation in primary intermediate infection of *Littorina littorea* in (a) Cusheen, (b) Lough Hyne, (c) Sandycove and (d) Whitegate. NS represent not sampled as Lough Hyne was only sampled & surveyed every second month. Error bars are 95% Clopper Pearson Confidence Intervals.

2.3.3 Effect of water quality

2.3.3.1 Non-trematode parasites

Prevalence of ciliates in *P. vulgata* significantly differed between non-reserve sites (χ^2=27.37, df=2, P<0.001) and the marine reserve (χ^2=36.64, df=3, P<0.001). Significantly lower prevalence of ciliates was recorded in the sites of high quality, Lough Hyne and Sandycove, relative to the site of moderate quality, Whitegate (Fig. 4). The prevalence of *P. ciliata* in the shells of hosts differed significantly between sites for *L. littorea* (χ^2=16.31, df=2, P=1), *N. lapillus* (χ^2=39.47, df=2, P<0.001) and *P. vulgata* (χ^2=29.08, df=2, P<0.001).

Cestode prevalence in *P. vulgata* differed between the three sites (χ^2=53.56, df=2, P<0.0001) and the marine reserve (χ^2=29.91, df=3, P<0.001). Highest (7.40%) and lowest (0.03%). cestode prevalences were recorded in the sites of moderate quality. Copepod prevalence in *P. vulgata* did differ between sites (χ^2=5.89, df=2, P=0.05) and the marine reserve (χ^2=5.88, df=3, P=0.05). Halacarid mites occurred in all hosts and significantly higher prevalences were recorded in the site of good quality, relative to sites of moderate quality (χ^2=12.80, df=2, P<0.05). Nematode prevalence was low for all hosts (<1%) and did not differ between sites or gastropod hosts.

2.3.3.2 Trematode parasites

Highest (n=7) and lowest (n=4) trematode richness were both recorded in sites of moderate quality. Seven trematode taxa were record in Cusheen, six in Sandycove, five in Lough Hyne and four in Whitegate (Table 2). Highest and lowest prevalences of trematodes were also recorded in sites of moderate quality, Cusheen and Whitegate, respectively (Fig. 4) (χ^2=99.56, df=2, P<0.0001). This was also the case with the subsampled dataset that was used for comparison with Lough Hyne (χ^2=77.34, df=3, P<0.0001). Trematode prevalence in *L. littorea* and *P. vulgata* significantly differed between the four sites (χ^2=77.39, df=3, P<0.0001). The prevalence of trematodes significantly differed between sites for all hosts, *L. littorea* (χ^2=23.91, df=2, P<0.001),

N. lapillus (χ^2=14.48, df=2, P<0.001) and *P. vulgata* (χ^2=27.37, df=2, P<0.001). Trematodes were more common in *N. lapillus* collected in Cusheen (P<0.001) and Sandycove (P<0.05) relative to Whitegate but overall had low prevalences (Fig. 4). *Nucella lapillus* is not discussed in further detail below as the gastropod species was rarely parasitised and by a limited number of trematode taxa (Table 2).

Littorina littorea was the most common primary intermediate host of trematode taxa (Table 2). Trematode richness in *L. littorea* did not significantly differ between sites. The highest richness (n=4) and lowest (n=2) number of trematode taxa were observed in sites of moderate quality (Table 2). The prevalence of primary intermediate infection in *L. littorea* did differ between sites, but the difference was only statistically significant between sites of moderate quality (χ^2=6.80, df=2, P<0.05) (Fig. 9). *Paramonostomum chabaudi* (Fig 5 (f)) was significantly more prevalent in the site of good quality relative to moderate quality sites (χ^2=6.45, df=2, P<0.05). *Podocotyle atomon* (Fig 5 (b)) was significantly less prevalent in the site of good quality (χ^2=12.30, df=2, P<0.05). *Renicola* spp. (Fig 5 (e)) and *C. lingua* (Fig 5 (a)) primary infections of *L. littorea* were more common in the site of good quality (Table 2) but did not significantly differ. Primary infection of *L. littorea* were grouped by the trematode's second intermediate host did vary in relation to quality status (Fig. 9). The highest number of groups were recorded in Sandycove and the lowest number in Whitegate (Fig. 9). Trematode taxa that infect molluscs were significantly more prevalent in Cusheen (χ^2=51.59, df=3, P<0.0001). Trematode taxa that encyst, external to host tissues, on hard substrates were only recorded in Sandycove (Fig 9).

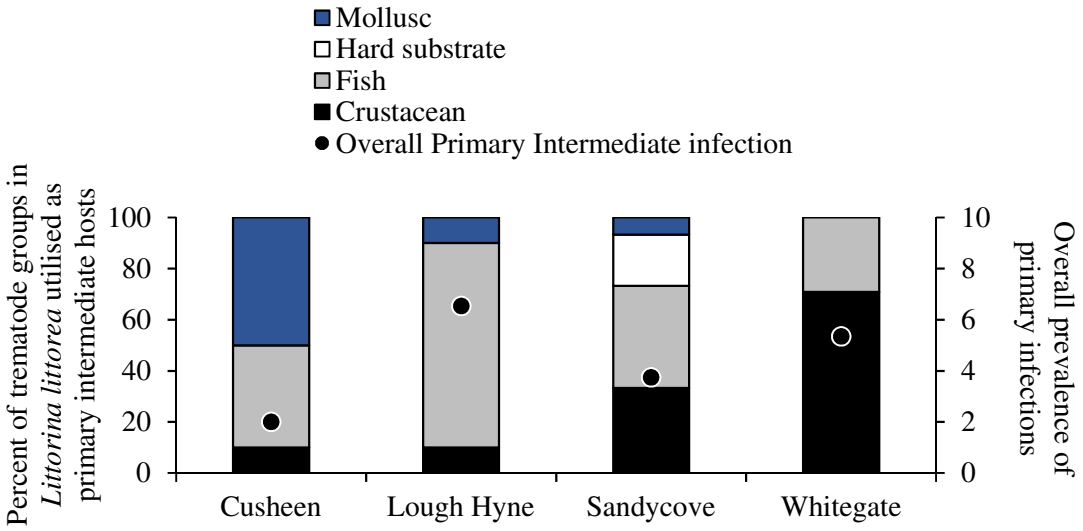

Fig. 9 The prevalence of trematode primary intermediate infections in *Littorina littorea* classified by their second intermediate host required in their lifecycle. The four primary intermediate host groups were fishes, crustaceans, molluscs, and hard substrates. Values were based on pooled total infections of all *L. littorea* per site.

The occurrence of trematode metacercariae in *L. littorea* significantly differed between sites of moderate quality, with lower prevalence recorded in Whitegate relative to Cusheen (χ^2 =51.38, df=3, P<0.0001). *Renicola* spp. metacercariae were the most common infection of *L. littorea* utilised as second intermediate host (Table 2). Prevalence of *Renicola* spp. metacercariae significantly differed between sites, with the highest and lowest prevalences recorded in sites of moderate quality (χ^2 =57.20, df=2, P<0.001) (Fig. 9). Prevalence of trematodes in *L. littorea* was higher in Cusheen relative to Sandycove (P<0.05) and Whitegate (P<0.001). When compared with Lough Hyne, highest and lowest prevalence of *Renicola* spp. was recorded in sites of moderate quality. Prevalence of trematodes in *L. littorea* in the marine reserve did not differ from other sites.

Patella vulgata was the most common second intermediate host for all sites and months sampled. *Echinostephilla patellae* prevalence differed between sites (χ^2=56.68, df=2, P<0.001) and Lough Hyne (χ^2=56.68, df=3, P<0.001) (Table 2 & Fig.

10). When compared with Lough Hyne the most significant differences were seen between high and moderate quality ($P<0.001$).

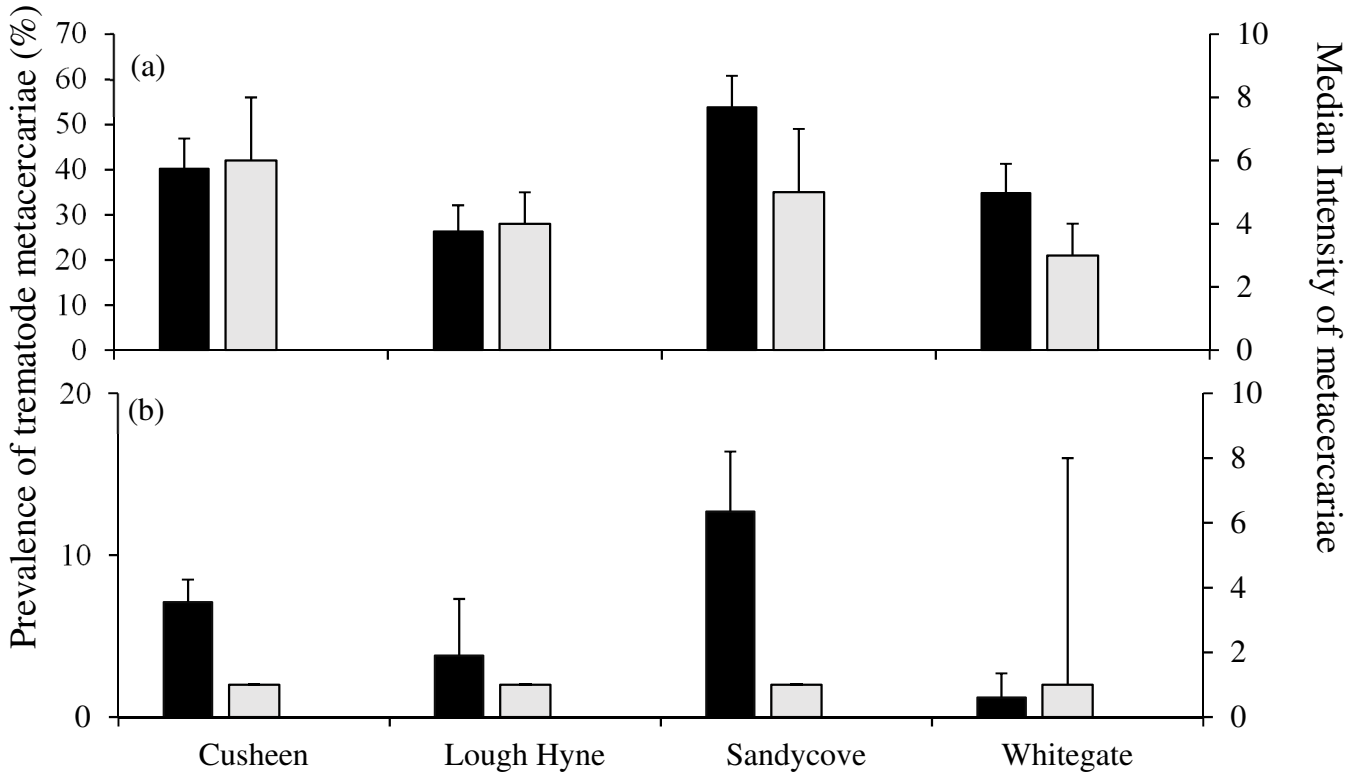

Fig. 10 (a) Prevalence (black) and median intensity (grey) of *Echinostephilla patellae* metacercariae in *Patella vulgata*. (b) Prevalence (black) and median intensity (grey) of *Renicola* spp. metacercariae in *Littorina littorea.* Error bars are 95% Clopper-Pearson 95% Confidence Intervals (CI). Prevalence and intensity estimated from all sampling events. Note the differing y-axes between (a) and (b).

2.3.4 Effect of local biotic factors and trematode infection

Echinostephilla patellae in *Patella vulgata* and *Renicola* spp. in *L. littorea* were the most common trematode taxa recorded in the study. For this reason, subsequent analysis in relation to local biotic factors focused on *E. patellae* and *Renicola* spp. metacercariae. Higher densities of *P. vulgata* were associated with higher prevalences of *E. patellae* in Sandycove ($r=0.12$, $df=408$, $P<0.05$). The positive association *Echinostephilla patellae* prevalence and *P. vulgata* density was observed in the three remaining sites but was not statistically significant. The size of *P. vulgata* also positively correlated with *E. patellae* prevalence in Sandycove ($r=0.24$, $df=438$, $P<0.0001$) and Whitegate ($r=0.25$, $df=359$, $P<0.0001$). Higher intensities of *E. patellae* metacercariae were recorded in larger *P. vulgata* ($r=0.15$, $df=1444$, $P<0.001$).

In all sites sampled, there was a negative correlation between *E. patellae* intensity with *P. vulgata* density, but this was statistically insignificant. In Cusheen, where *Mytilus* spp. were common (Table 3), the intensity of *E. patellae* significantly and negatively correlated with the bivalve's density ($r=-0.13$, $df=391$, $P<0.05$). The negative correlation was found in all remaining sites but was not statistically significant. Perennial alga percentage cover negatively correlated *E. patellae* intensity with but was only statistically significant in Lough Hyne ($r=-0.20$¸ $df=212$, $P<0.05$). Increased coverage of ephemeral algal coverage negatively correlated with *P. vulgata* shell height ($r=-0.07$, $P<0.05$).

Renicola spp. metacercariae positively correlated with their host *L. littorea* density ($r=0.10$, $P<0.001$). No significant correlations were found between the remaining ambient biota and *Renicola* spp. metacercariae in *L. littorea*. More trematodes were recorded in larger *L. littorea. Cryptocotyle lingua* positively correlated with *L. littorea* shell height ($r=0.10$, $P<0.001$), width ($r=0.09$, $P<0.001$), whole weight ($r=0.09$, $P<0.001$), tissue weight ($r=0.08$, $P<0.001$) and negatively with *L. littorea* density ($r=-0.33$, $P<0.001$). Prevalence of *C. lingua* positively correlated with ephemeral algal species percentage cover ($r=0.14$, $P<0.001$). It can be seen from the nMDS that infection status of *P. vulgata* had a stronger effect on the grouping than site (Fig 11 a & b).

Table 3 Recorded biota for in study sites in 0.25 m ^{2} quadrats from May 2014 until April 2015. Abundance was calculated from a total of 117 quadrats in Cusheen, 49 in Lough Hyne, 118 in Sandycove and 111 in Whitegate. Units are represented as 1 is presence/absence, 2 is SACFOR, 3 is counts per 1 m ^{2}, and 4 is percentage cover.

Biota recorded	Functional group	Unit	Cusheen	Lough Hyne	Sandycove	Whitegate
Actinia equina (Linnaeus, 1758)	Anemone	1	0.06	0.03	0.27	0.02
Anemonia viridis (Forsskål, 1775)	Anemone	1	0.01	0.01	0.17	0.00
Austrominius modestus (Darwin, 1854)	Barnacle	2	0.69	0.22	0.00	0.64
Chthamalus montagui Southward, 1976	Barnacle	2	0.31	0.02	0.02	0.15
Chthamalus stellatus (Poli, 1791)	Barnacle	2	0.02	0.01	0.00	0.03
Semibalanus balanoides (Linnaeus, 1767)	Barnacle	2	0.13	0.00	0.00	0.03
Cancer pagurus Linnaeus, 1758	Decapod crab	1	0.00	0.02	0.03	0.02
Carcinus maenas	Decapod crab	1	0.02	0.02	0.03	0.21
Pagurus sp. Fabricius, 1775	Decapod crab	1	0.01	0.00	0.03	0.03
Littorina littorea	Host gastropod	1	0.75	0.19	0.54	0.65
Nucella lapillus	Host gastropod	3	0.65	0.09	0.62	0.65
Patella vulgata	Host gastropod	3	0.95	0.37	0.86	0.76
Ectocarpus sp. Lyngbye, 1819	Ephemeral alga	4	0.01	0.04	0.00	0.01
Ulva spp. Linnaeus, 1753	Ephemeral alga	4	0.00	0.11	0.14	0.11
Mytilus spp.	Paratenic host	3	0.72	0.05	0.03	0.07
Gibbula spp. Risso, 1826	Paratenic host	1	0.49	0.12	0.58	0.57
Littorina fabalis (Turton, 1825)	Paratenic host	1	0.07	0.03	0.06	0.11
Littorina obtusata (Linnaeus, 1758)	Paratenic host	1	0.23	0.03	0.25	0.43

Table 3 Continued

Biota recorded	Functional group	Unit	Cusheen	Lough Hyne	Sandycove	Whitegate
Littorina saxatilis (Olivi, 1792)	Paratenic host	1	0.42	0.06	0.04	0.05
Ascophyllum nodosum (Linnaeus) Le Jolis, 1863	Perennial alga	4	0.26	0.23	0.02	0.17
Fucus serratus Linnaeus, 1753	Perennial alga	4	0.09	0.01	0.49	0.75
Fucus spiralis Linnaeus, 1753	Perennial alga	4	0.01	0.04	0.08	0.03
Fucus vesiculosus Linnaeus, 1753	Perennial alga	4	0.21	0.16	0.21	0.17
Laminaria sp.	Perennial alga	4	0.00	0.01	0.02	0.03
Spirobranchus sp. Blainville, 1818	Polychaete	1	0.07	0.07	0.22	0.41
Spirorbis sp. Daudin, 1800	Polychaete	1	0.10	0.15	0.36	0.57
Chondrus sp. Stackhouse, 1797	Turf alga	4	0.03	0.01	0.14	0.05
Cladophora sp. Kützing, 1843	Turf alga	4	0.05	0.20	0.02	0.01
Mastocarpus sp. Kützing, 1843	Turf alga	4	0.03	0.02	0.25	0.07

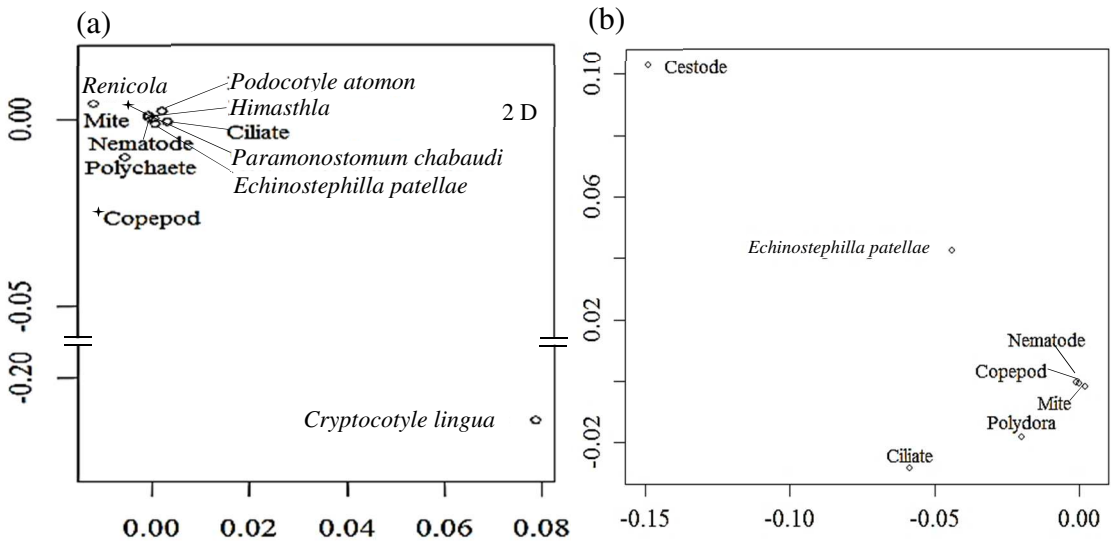

Fig. 11 Nonmetric multi-dimensional scaling (nMDS) plots comparing the parasite species composition in (a) *Littorina littorea* and (b) *Patella vulgata* among the four rocky shores in SW Ireland sampled 2014-2015. Plots are based on Jaccard similarities and presence/ absence data. Stress values of all non-metric multidimensional scaling ordinations (nMDS) were below 0.2 and so were considered to represent data accurately in the ordination.

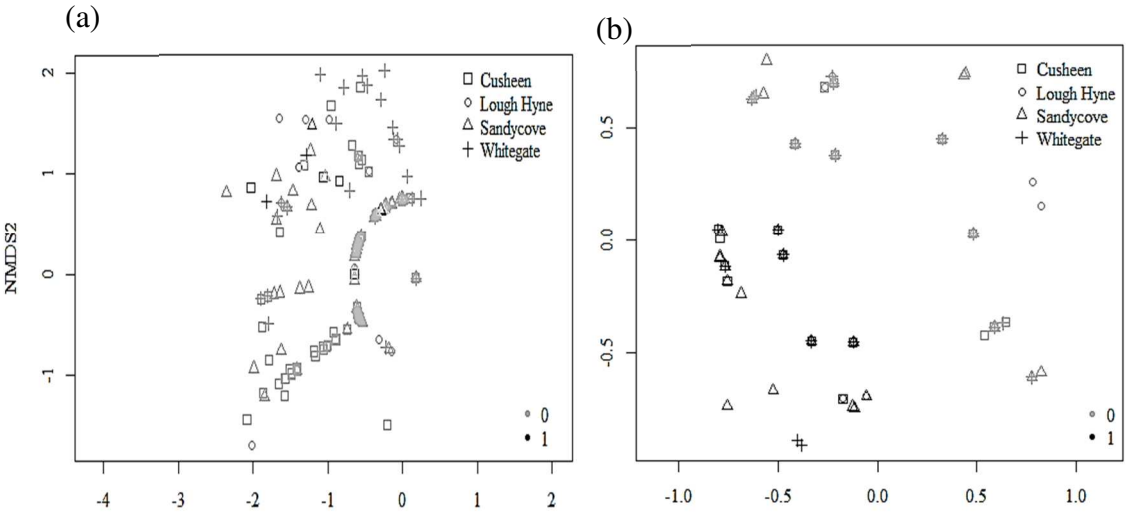

Fig. 12 Nonmetric multi-dimensional scaling (nMDS) plots of (a) *Littorina littorea* and (b) *Patella vulgata* among the four rocky shores in SW Ireland sampled 2014-2015. Symbols denote sites sampled and colours deonote the presence (black) and absence (grey) of trematode metacercariae of (a) *Renicola* spp. or (b) *Echinostephilla patellae.* Plots are based on Jaccard similarities and presence/ absence data. Stress values of all non-metric multidimensional scaling ordinations (nMDS) were below 0.2 and so were considered to represent data accurately in the ordination.

2.4. DISCUSSION

This study illustrates the complexity of the response of recorded parasite taxa to quality. There was no clear pattern in parasite taxa richness between sites related to WFD water quality classification. Ciliates and cestodes were less common in the sites of high quality. Highest trematode occurrence and richness were observed in the sites of moderate quality. The functional groups of trematodes utilising *L. littorea* as a primary intermediate host also varied in relation to quality classifications, with higher richness found in the site of good quality. However, the highest and lowest prevalence of primary intermediate infection of *Littorina littorea* were observed on shores of moderate quality. Trematode taxa *Echinostephilla patellae* and *Renicola* spp. were more prevalent on shores of good quality.

Surrounding intertidal biota were expected to decrease intensity of trematode metacercariae, as this has been demonstrated in multiple laboratory trials (Hopper *et al.,* 2008, Thieltges *et al.,* 2008, Prinz *et al.,* 2009, Studer *et al.,* 2013, Welsh *et al.,* 2014). The effect of biota on trematode infection may be context specific. The association between host density and prevalence of *E. patellae* and *Renicola* spp. was not consistent between sites or hosts, *P. vulgata* and *L. littorea* respectively. Furthermore, the effect of surrounding biota on trematode transmission would likely depend on the abundance of the biota. In this study, the correlation between decreased *E. patellae* intensity and increased number of *Mytilus* spp. was only significant on the shore where the bivalve was abundant. A possible reason for the decreased intensity of *E. patellae* in *P. vulgata* associated with patches of *Mytilus* spp. is that *E. patellae* cercariae may be physically disturbed in the water column or infect the bivalve instead of *P. vulgata* (Thieltges *et al.,* 2008, Prinz *et al.,* 2009, Welsh *et al.,* 2014). Intertidal *Mytilus* spp. have been recoded to serve as second intermediate hosts for *Echinostephilla patellae* (Prinz *et al.,* 2009; 2010) and can physically interfere with the swimming and subsequent infection of cercariae through the action of filter feeding (Prinz *et al.,* 2009). *Patella vulgata* and *N. lapillus* were the most and least parasitised host species of the study. *Nucella lapillus* was predominately parasitised by the

burrowing polychaete, *P. cilata* but rarely by other parasite taxa. This difference is likely be due to the immunocompetence of the gastropods and differing encounter rates between the gastropods and their parasite taxa. *Nucella lapillus* within the size range sampled and geographic region of the study, have been recorded to be commonly infected by a range of parasite taxa (Ebling *et al.,* 1964, Crothers, 1985, Prinz*et al., et al.,* 2010). Final hosts of trematodes are considered a determining factor in trematode distribution and prevalence (Kube *et al.,* 2002, Hechinger & Lafferty, 2005, Feis *et al.,* 2015). Thus, the observed difference in trematode occurrence in *N. lapillus* relative to *P. vulgata* may be due to differing encounter rates with avian final hosts. Evidence to support this suggestion is the preference of the Eurasian oystercatcher *Haematopus ostralegus* (Linnaeus, 1758), a final host of the trematode *Echinostephilla patellae,* to consume *Patella* spp. over *N. lapillus.* The preference is attributed to the unpalatable secretions that *N. lapillus* produces to bore into molluscs' shells (Feare, 1970, Kollien, 1996, Hawkins, 1999).

Littorina littorea was parasitized by the highest number of trematode taxa. Its trematode community structure was similar to that of the Atlantic and Wadden Sea; *C. lingua* was the dominant species utilsing *L. littorea* as a primary intermediate host. In terms of abundance it is followed by *Renicola* spp., *Himasthla* spp., *P. chabaudi* and *P. atomon* (James 1968a, Thieltges *et al.,* 2006, Prinz *et al.,* 2010a). A higher prevalence of mollusc and crustacean infecting primary intermediate infections was recorded in Cusheen and Whitegate. This, may be due to the higher abundance of the trematodes' *Renicola* spp., and *P. atomon* second intermediate hosts, *C. maenas* and *Mytilus* spp., observed on Cusheen and Whitegate, respectively.

The recorded abundance of trematodes from the families Echinostomatidae (e.g. *E. patellae*, *Renicola* spp) and Heterophyidae (*C. lingua*), and low number of co-infections could be due to competitive interactions between trematode taxa within the host. Taxa within these families have rediae, "a soldier caste", that consume heterospecifics and conspecifics from other infections (Hechinger *et al.,* 2011, Garcia-Vedrenne *et al.,* 2016; 2017). The "soldier" caste of treamtode rediae were observed

within primary intermediate hosts and have been recorded to differ from reproductive rediae in their morphology, spatial distribution, and behaviour (Hechinger *et al.,* 2011). *Echinostephilla patellae* and *C. lingua* have redial stages, however, competitive interactions between their rediae and other trematode infections have yet to be observed (Kollien 1996, Rees 1934, Stunkard 1930). This study provides a conservative estimate of the trematodes *Paramonostomum chabaudi* and *Parorchis acanthus* as their metacercariae enycyst in the external environment and were not observed.

Echinostephilla patellae was the most common trematode of the study. Prevalences and intensities of *E. patellae* recorded in this study were higher than in previous work (see Table 4). This is likely due to the different shore heights sampled, a different examination method and the inclusion of metacercariae in counts (see Table 4). This study and work by Prinz *et al.,* 2010a examined the whole body of *P. vulgata* while Firth *et al.,* 2017 largely examined gastric smears and so may have encountered more parasites as more tissue was examined.In contrast to this study, Firth *et al.,* 2017 and Prinz *et al.,* 2010a did not include metacercariae in their prevalence calculations of *E. patellae*.

No primary infections of *E. patellae* were recorded in this study, but they were observed on other shores during the time period (pers. obs.). The absence of primary infection *E. patellae* was likely due to the fact that *P. vulgata* outside of rock pools from the low shore were sampled. Previous work has recorded higher prevalence of *E. patellae* primary infections in rock pools (Crewe, 1951, Thomas, 1965, Copeland *et al.,* 1987). However, the opposite pattern has been reported by Kollien, (1996) and some shores sampled by Firth *et al.,* (2017).

Table 4 Summary of results of previous studies and methods of assessing *Echinostephilla patellae* abundance in *Patella* spp. arranged in chronological order

Location	Method	Life stage	n	Prevalence	Reference
Great Britain	Dissection	Redial	3,631 3,777	4%	Crewe, 1947, 1951
Inner Farne, Northumberland, UK	Dissection	Redial	1,750	13.5%	Thomas, 1965
Cardigan Bay, Southwest UK	Dissection	Redial	503	3.77%	James 1968a
Portavogie Harbour, Northern Ireland.	Squash preparations	Redial	200	91.5%	Copeland *et al.,* 1987
St. Andrews, east coast Scotland, UK	Macroscopic examination and dissection	Redial	1,160	32%	Kollien, 1996
	Dissection	Metacercariae	400	67%	Kollien, 1996
West Ireland Clare Island	Macroscopic examination	Redial	3,350	12%	Delany 2000
Southwest coast Ireland	Squash preparations	Redial	500	3.6+2.4%	Prinz *et al.,* 2010a
East and West coast Ireland	Visual examination & Gastric smears	Redial	1,580	28%	Firth *et al.,* 2017

Prevalence and intensity of trematode taxa varied between sampling events but not in relation to the season. Temporal variation in *E. patellae* metacercariae prevalence or intensity did not exhibit a distinct peak but was higher in the winter months. No seasonal peak in prevalence of trematodes in *L. littorea* utilised as a primary or secondary host was observed. Similar patterns have been recorded in metacercariae of other trematodes (Sindermann & Farrin, 1962, Robson & Williams, 1970, Martin, 1971, Lauckner, 1980). Patterns in seasonal variation of *E. patellae* or *Renicola* spp. metacercariae could be confounded by pre-existing infections from multiple years within a single gastropod. Nikolaev *et al.,* (2006) found that metacercariae of trematodes within superfamily Echinostomatidae in *Mytilus edulis* were viable years after initial infection.

The first record of the adult worm *Proctoeces* sp. in *P. vulgata* and *N. lapillus* in Ireland was also of note. *Proctoeces* spp. have previously recorded in *Patella* spp. in the Mediterranean (Le Pommelet *et al.,* 1997) and in *N. lapillus* in New England had low prevalence but high intensities of adult worms in the kidneys (Pondick, 1983). In this study *Proctoeces* occurred at one site, Cusheen, which had a high density of *Mytilus* sp., the preferred primary host of *Proctoeces* sp. (Lang & Deunis, 1976).

Trematode taxa recorded in the study are considered relatively harmless to humans (Loos-Frank 1967, Loos-Frank 1969). To the author's knowledge, the practice of consuming uncooked marine invertebrate taxa screened in this study is rare in Ireland. Thus, food-borne infection by trematode taxa recorded is improbable but possible. Infection by *Cryptocotyle lingua*, may occur via the consumption of the its second intermediate host, bony fish, rather than its primary intermediate host *L. littorea* (Cummins *et al.,* 2002, Stunkard 1930). *Cryptocotyle lingua* infects fish-consuming-mammals and has been detected in coastal otters *Lutra lutra* in Ireland (McCarthy *et al.,* 1993) and humans in Greenland and Alaska (Babbott *et al.,* 1961, Rausch *et al.,* 1967). Infection was not considered to be harmful and can be avoided with sufficient cooking or freezing of fish, prior to consumption (Babbott *et al.,* 1961, Rausch *et al.,* 1967, BIOHAZ 2010, Borges *et al.,* 2015).

The distribution of trematode taxa, recorded in this study, varied over time and in relation to quality. This study found site-specific effects of biotic communities on the intensity of trematode metacercariae. To provide baselines or accurate predictions of trematodes to future changes, the taxa must be studied in the field or under biologically realistic conditions.

Potential future changes in intertidal communities such as climate change and associated stressors may further impact on gastropod: trematode dynamics (Thompson *et al.,* 2002, Poulin, 2006, Poulin & Mouritsen, 2007). One such change is the rise in global ocean temperature of 0.10 °C, from the surface to a depth of 700m, since 1961 with a predicated maximum increase of 4 °C by the year 2100 is (Callaway *et al.,*

2012). Increases in temperature have already occurred in the seas around Ireland, as rises of 0.3-0.4 ℃ per decade have been recorded since the 1980s (Dunne *et al.,* 2013). In an Irish context, sea temperature, mean annual temperatures of 1–1.6°C and storm severity are all predicted to increase (Nolan, 2015). The predicted changes may facilitate the transmission of larval stages of trematodes. Metanalysis by Poulin (2006) indicated that a 10℃ increase could results in up to a 200-fold increase of cercarial emergence from first intermediate hosts. The emergence of cercariae vary within the same infection, and between trematode taxa and first intermediate host taxa (Poulin, 2006). However, the predications on the response of parasite taxa to predicated changes have largely focused on the effect of temperature on a single trematode taxon (Poulin 2006, Marcogliese 2016). Thus, accurate predictions of parasite taxa's response to predicated climate change are difficult to make, due to the gaps in the knowledge, the inherent complexity of the host-parasite relationship and the neglect of environmental stressors outside of predicted anthropogenic warming (Harvell *et al.,* 1999, Ward & Lafferty 2004, Poulin, 2006, Marcogliese, 2016). In particular the relationships need to be evaluated in relation to abundant trematode such as *Echinostephilla patellae*, further work should consider its ecology and the effect of its metacercariae on *P. vulgata.*

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CHAPTER 3: Distribution of parasites of the European Limpet (*Patella vulgata*) in a marine reserve

ABSTRACT

Marine reserves are instrumental in the implementation of successful marine conservation or commercial fisheries management. In the literature to date, some work has been done on the dynamics of pathogens and parasites within marine reserves. Marine reserves can encourage parasitic infection, as within their boundaries, biota can attain larger sizes and densities, which can favour increased parasite load and enhanced transmission. This study was conducted to examine whether parasite communities of a common grazer, the limpet *Patella vulgata*, varied inside and outside a marine reserve in Southwest Ireland. The roles of coastal configuration, host density, and the location of populations inside and outside of the reserve were considered in relation to distribution of parasites in *P. vulgata*. Parasitism was commonly observed inside and outside the reserve. Over half of *P. vulgata* screened were infected by one or more of five parasite taxa from four phyla: Annelida, Arthropoda, Ciliophora and Platyhelminthes. The most common parasite taxa were trematode metacercariae of *Echinostephilla patellae* and the ciliate *Leiotrocha patellae*. Large variation in parasite distribution over small spatial scales was recorded, but generally, higher occurrence and burdens of trematode metacercariae were recorded in sheltered sites and with slower water currents, indicating the significance of water movements in parasite transmission and retention. The observed similarity in parasite communities within and outside the reserve is likely to result from the wide dispersal range of final avian hosts, which extends to all sites sampled. This study indicates that other site-specific local factors such as host density, coastal configuration and host interaction have a greater influence on parasites than location within a reserve alone.

3.1 INTRODUCTION

Parasitic taxa are generally ignored in conservation biology, despite their ubiquity and potential ecological relevance (Poulin 1999, Dobson *et al.,* 2008). The neglect of parasites has been attributed to a general distaste for and the difficulty in studying cryptic taxa (Windsor 1995, Nichols *et al.,* 2011, Gómez *et al.,* 2013). Parasites are increasingly recognised to play a role in community structure and functioning (Marcogliese 2004, Poulin & Mouritsen, 2006). The effect of anthropogenic impacts, such as declining biodiversity and predicted climatic change, on marine parasites is largely unknown (Ward *et al.,* 2004, Marcogliese, 2016, Thieltges *et al.,* 2016). Despite this, there is speculation that the extent and severity of infection by marine parasites will increase in response to predicted changes in global warming, host range and abundance (Poulin 2006, Poulin & Mouritsen*,* 2006).

Marine protected areas (hereafter MPAs) have a remit to protect biodiversity or provide a refuge for marine biota from commercial harvesting and anthropogenic impacts. MPAs are a common management tool in conservation biology and are defined by the IUCN as "(a) clearly defined geographical space, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values" (Day *et al.,* 2012). The most restricted or protected area is a reserve, within which no extractive activities are permitted (Lester and Halpern, 2008). Within a reserve's boundaries, a species' abundance or biomass can rapidly increase (Gell & Roberts, 2003, Byers, 2005, Lester & Halpern, 2008). This may benefit local fisheries if the increasingly abundant or larger sized individuals of commercially exploited species leave the boundaries of the reserve, an effect known as "spillover", and can then be harvested from nearby unprotected areas (Gell and Roberts, 2003).

Generally, the effects of marine reserves on commercial, especially on vertebrate species, are well understood (Murray *et al.,* 1999, Gell & Roberts, 2003, Halpern *et al.,* 2009). Some attention has been paid to non-commercial or invertebrate species (Lubchenco *et al.,* 2003, Fenberg *et al.,* 2012) and parasite dynamics within marine reserves (Wootton *et al.,* 2012). Within a reserve, a host species could increase in abundance, density, and size (Gell & Roberts, 2003, Byers, 2005, Lester & Halpern, 2008). According to classic epidemiological theory, these changes may favour successful transmission of parasites or disease outbreak (Kermack & McKendrick, 1927). This has occurred in terrestrial systems (Lebarbenchon *et al.,* 2006). The increased abundance of a parasite within the protected area can result in a parasite "jumping" to new host species, that gather in large numbers e.g. shorebirds (Lebarbenchon *et al.,* 2006). To ensure conservation of the marine reserves' target species it is critical to understand parasite dynamics within their boundaries and whether these dynamics can inhibit its objectives. A marine reserve could act as a parasite reservoir if a parasite is permanently maintained within the reserve's boundaries and is able to transmit to nearby host populations or "spillover" into the surrounding area (Haydon *et al.,* 2002).

At present, work on pathogens in MPAs is fragmented, with a limited number of locations (n=10) and taxa (n=25) considered (Table 1). The limited number of empirical studies on parasites in MPAs, make generalisations of parasites within reserves difficult and have provided conflicting results (see Table 1). The potential for a marine reserve to facilitate parasitic infections is likely dependent on several factors, the life history of the parasite taxa, their hosts, and the characteristics and location of the reserve itself. The location of the reserve, its water currents and degree of exposure may affect parasite communities within its boundaries. In the Northern Hemisphere at Lundy Island Marine Reserve, UK, European *Homarus gammarus* (Linnaeus, 1758) experienced a higher incidence of shell disease within protected areas (Wootton *et al.,* 2012, Davies *et al.,* 2015). While *Jasus edwardsii* (Hutton, 1875) experienced a lower incidence of tail fan necrosis in New Zealand marine reserves (Freeman & MacDiarmid, 2009). Work, in the Great Barrier Reef and Mediterranean Sea, found that protected areas had partial or no effect on coral

pathologies (Ternengo *et al.,* 2009, Lamb *et al.,* 2016). In France, monogenean communities of the White Seabream *Diplodus sargus* (Linnaeus, 1758) did not differ between protected and unprotected areas (Sasal *et al.,* 2004).

Table 1 List of studies generated by the database ISI web of Science ™ for studies on parasites or pathogens within MPAs or marine reserves. Magnitude of effect of the reserve (if any) on parasite taxa an increase in infection parameters is represented as +, a decrease as – and no effect as N.

Parasite	Host	Site	Effect of reserve	Magnitude	Reference
Acanthocephalan three species of trematode, nematode.	Bucchich's goby *Gobius bucchichii* Steindachner, 1870 n=396	Cerbère-Banyuls marine reserve, in the southeast of France	Individuals within protected zones were on average larger, older and had higher prevalence of parasites.	+	Sasal *et al.,* 1996
The monogenean *Lamellodiscus* spp., Johnston & Tiegs, 1922	White seabream *Diplodus sargus* (Linnaeus, 1758) n=104		No modification in global parasite community. Only one, *L. elegans*, increased its abundance in the protected area relative to fished zones.	+	Sasal *et al.,* 2004
Bacterial wasting disease.	Purple sea urchin n=27, 456 *Strongylocentrotus purpuratus* (Stimpson, 1857) Red sea urchin n=19, 338 *Mesocentrotus franciscanus* (A. Agassiz, 1863)	Southern Californian rocky reef	Disease was rare at reserve sites but common at fished sites.	-	Behrens and Lafferty, 2004
Bacteriae *Rickettsia* spp.	Abalone *Haliotis* spp. (Linnaeus, 1758)	NA-(Modelled Californian ecosystem)	A deterministic model of microparasite infection found that the yield of abalone decreased in response to *Rickettsia* spp.	+	McCallum *et al.,* 2005

Table 1 Continued

Parasite	Host	Site	Effect of reserve		Reference
Monogenean and Digenean trematodes, cestodes, nematodes, acanthocephalans, and copepods.	Bicolor chromis n=25 *Chromis margaritifer* Fowler, 1946 Blackbar devil n=50, *Plectroglyphidodon dickii* (Liénard, 1839) Arc-Eye Hawkfish n=49 *Paracirrhites arcatus* (Cuvier, 1829) Whitecheek Tang n=26 *Acanthurus nigricans* (Linnaeus, 1758) Two-spot red snapper n=38 *Lutjanus bohar* (Forsskål, 1775)	Northern Pacific	Generally, parasite richness, intensity and prevalence were higher for all fish species in the non-fished site.	+	Lafferty *et al.,* 2008
Handling-related bacterial infection.	Spiny lobster n=7, 466 *Jasus edwardsii*	New Zealand marine reserve	Lobsters outside the reserve were significantly more affected by tail fan necrosis than lobsters within the reserve.	-	Freeman and MacDiarmid, 2009
Skeletal eroding band, brown band disease and growth anomalies.	Reef building coral Acroporidae and Poritidae	Republic of Palau, located in the western Pacific Ocean	Skeletal eroding band was 2-fold higher within MPA.	+	Page *et al.,* 2009

Table 1 Continued

Parasite	Host	Site	Effect of reserve		Reference
Aspidogastrean, Digenean and monogean trematodes, cestodes, nematodes, acanthocephalans and crustaceans. Fungal shell disease.	Common two-banded seabream *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817) n=72 Striped red mullet n=68 *Mullus surmuletus* Linnaeus, 1758 Common pandora n=47 *Pagellus erythrinus*, (Linnaeus, 1758) Forkedbeard n=40 *Phycis phycis,* (Linnaeus, 1766) Red scorpionfish n=57 *Scorpaena scrofa* Linnaeus, 1758 Peacock wrasse n=58 *Symphodus tinca* (Linnaeus, 1758)	Bonifacio Strait Marine Reserve, Mediterranean Sea	Parasite distributions were not influenced by the protection status of the site. parasite life traits history remained a fundamental determinant in the community composition	N	Ternengo *et al.,* 2009
Fungal shell disease.	European lobsters n=666 *Homarus gammarus* (Linnaeus, 1758)	Lundy Island, United Kingdom	Increased host abundance, injury and shell disease detected in the no take zone of the reserve.	+	Wootton *et al.,* 2012

Table 1 Continued

Parasite	Host	Site	Effect of reserve		Reference
Digenean trematode *Proctoeces lintoni* (Siddiqi & Cable, 1960)	Keyhole limpet n=202 *Fissurella latimarginata* G. B. Sowerby I, 1835 Intertidal mussels n=2,509 *Perumytilus purpuratus* (Lamarck, 1819) Clingfish n=71 *Sicyases sanguineus* Müller & Troschel, 1843	Central Chile	Higher number of infection recorded in the MPAs in limpets and mussels	+	Loot *et al.,* 2005
P. lintoni	*F. latimarginata* n=59	Central Chile	Higher number of eggs recorded in the MPAs	+	Loot *et al.,* 2008,
Gill parasites Maxillopoda, Malacostraca, Pinnotherid pea crab, Monogenean and Digenean trematodes.	Bilagay n=100 *Cheilodactylus variegatus* Valenciennes, 1833 Jerguilla n=120 *Aplodactylus punctatus* Valenciennes, 1832 Sea urchin n=149 *Loxechinus albus* (Molina, 1782) *F. latimarginata* n=133		One significant effect for one parasite species; *Encotyllabe* sp. (Diesing, 1850) from the gills of *C. variegatus*		Wood *et al.,* 2013
P. lintoni	*F. latimarginata* n=568		Increased parasitism within the marine reserves	+	Aldana *et al.,* 2013

Table 1 Continued

Parasite	Host	Site		Effect of reserve		Reference
Black band disease, skeletal eroding band, brown, band disease, white syndromes, atramentous necrosis or growth anomalies.	Reef-building corals n=36, 104	Great Reef Park	Barrier Marine	The marine reserves were only partially effective in mitigating disease prevalence	-	Lamb *et al.,* 2016

Conflicting results can even be found within the same protected area (Table 1). *Perumytilus purpuratus* and *F. crassa* were more commonly infected with the trematode *P. lintoni* in Chilean MPAs (Loot *et al.,* 2005). However, further work in the areas found no difference in the parasite communities of *F. latimarginata*) and sea urchins *L. albus* between protected and unprotected zones (Wood *et al.,* 2013).

Coastal configuration is known to affect shore exposure, water currents and intertidal communities (Jessopp *et al.,* 2008, Burrows, 2012). Wave exposure is known to be a determining factor in the structure of intertidal communities on rocky shores (Burrows (Burrows *et al.,* 2008). Increasing exposure of rocky shores leads to a shift from macroalgae to filter feeders in intertidal communities (Thompson *et al.,* 2002). Changes in potential host communities will likely change respective parasite communities.

This study was undertaken to examine parasite infection of a non-commercial invertebrate species in a marine reserve. Lough Hyne is the only statutory marine reserve in the Republic of Ireland. Parasites have been previously recorded within the reserve, in the form of parasitic copepods and barnacles in decapod crabs (Renouf 1932, Costello & Myers, 1989, Minchin, 1997, De Grave & Holmes, 1998, Lyons, 2011). Trematodes have been recorded in barnacles, *Chthamalus montagui* and *C. stellatus* (O'Riordan *et al.,* 1992), decapod crabs (Lyons, 2011), bivalves, *Mytilus* spp. (Lynch *et al.,* 2014) and sea squirts *Ascidiella aspersa* (Müller, 1776) (Lynch *et al.,* 2016). However, the present study aimed to explicitly compare parasite infections within and outside the reserve.

Patella vulgata was chosen as model species to screen as a host for parasite abundance, as it frequently harbours parasite taxa in Ireland (Copeland *et al.,* 1987, Prinz *et al.,* 2010a, Firth *et al.,* 2017). Furthermore, it is a keystone grazer and an abundant gastropod on rocky shores, including those within Lough Hyne (Little *et al.,* 1988, Crowe *et al.,* 2000, Moore *et al.,* 2007). *Patella vulgata* also has high site fidelity, has

a foraging range of up to 1 metre (Wright & Hartnoll, 1981) and is in close contact with the substrate (Orton, 1929, Little *et al.,* 1988). These factors may enable the detection of variation in parasite communities over a small spatial scale. Although, *P. vulgata* is not commercially harvested in Ireland, on a global scale, many patellid limpet stocks are overexploited (Borges *et al.,* 2015). Simulated exploitation of *P. vulgata* induced changes in the life history of the gastropod. *Patella vulgata* populations at two locations in southwest England had the largest individuals systematically removed for 18 months. In areas of lower density of large individuals sex change was induced at smaller sizes. indicating that the gastropod may be susceptible to harvesting (Borges *et al.,* 2015).

Lough Hyne is sheltered, has restricted tidal exchange with outer waters and can retain and self-seed with invertebrate larvae (Johnson *et al.,* 1995, Jessopp *et al.,* 2007, Jessopp & McAllen, 2008). Larvae of the saddle oyster *Anomia ephippium* and the crustacean *Austrominius modestus* were, imported into the marine reserve and were more abudnant withithan in adjacent shores (Jessopp & McAllen, 2007; 2008). It is speculated that the unusual coastal configuration of Lough Hyne, in combination with less anthropogenic disturbance could facilitate parasitic communities of *P. vulgata* within Lough Hyne. It was hypothesised that a greater incidence and richness of parasite taxa would occur within the boundaries of the reserve.

3.2. MATERIALS AND METHODS

3.2.1 Sites

The study area encompassed ten rocky shores along the southwest coast of Ireland (see Table 2). Toe Head Bay was the most easterly site and the Western shore of Lough Hyne marine reserve was the most westerly (Fig. 1). Lough Hyne, covers an area of 0.8km^2 and has a limited tidal range (ca. 1m) (Ebling *et al.,* 1960). Lough Hyne has restricted tidal exchange with outer waters due to the dimensions of the connecting

channel. The channel, called the "rapids", is approximately 5m deep and 25m wide. Within the channel, an area rapidly shallows, creating a sill. During a flood tide, water must overcome the sill to enter Lough Hyne. Currents in the "rapids" reach speeds greater than $3ms^{-1}$ during the flood tide (Bassindale *et al.,* 1948, Bassindale *et al.,* 1957, Ebling *et al.,* 1960). It takes an estimated 41 days for water to be flushed from Lough Hyne (Johnson *et al.,* 1995). Water enters the reserve and moves northward in an anticlockwise gyre, slowing as it reaches the west side of Lough Hyne (Bassindale *et al.,* 1957). Water continues to slow as it leaves the Southern basin and moves towards the North basin (Bassindale *et al.,* 1957, Greenwood *et al.,* 2001, Bell & Barnes, 2002). The other sites, Tralispean, Tragumna and Toe Head Bay, were chosen to compare with Lough Hyne, as they are close, but not connected, to the reserve (Fig. 1). The low shore was sampled at all sites to allow comparison with the reserve as intertidal biota are concentrated within in its limited tidal range (Little *et al.,* 1988, Little, 1991).

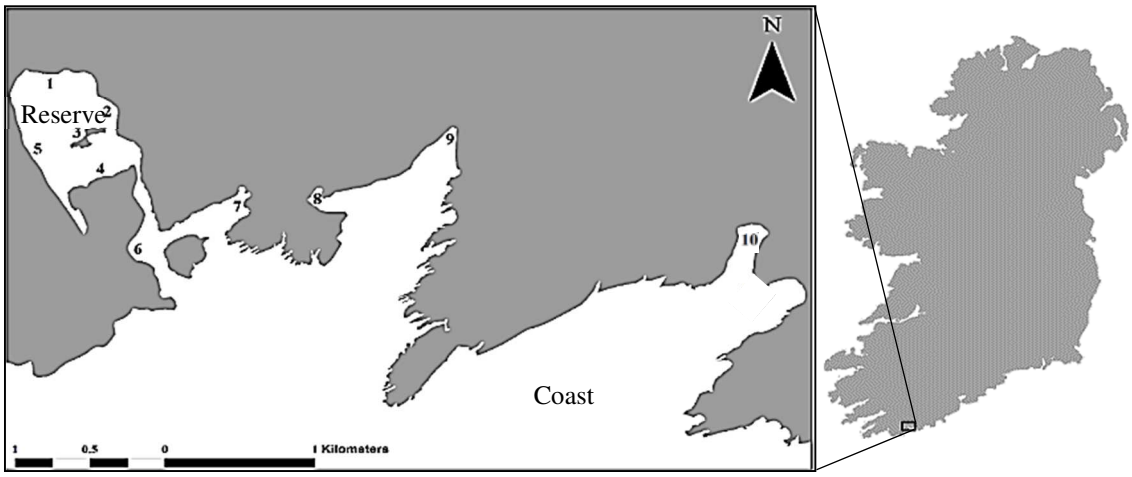

Fig. 1 Map showing ten sites along the south coast of Ireland where 30 *Patella vulgata* (b) were sampled from in June 2015. 1 Lough Hyne North, 2 Lough Hyne East, 3 Castle Island, 4 Lough Hyne South, 5 Lough Hyne West, 6 Barloge, 7 Tranabo, 8 Tralispean, 9 Tragumna and 10 Toe Head Bay. Adapted from Environmental Protection Agency Water Quality shapefile.

Table 2. Descriptions of study sites (modified from Bassindale *et al.,* 1957 & Ebling *et al.,* 1960), with Ballantine scale added (Ballantine, 1961)

Site	GPS	Ballantine Scale	Site description
1 Northern Shore	51°30'21''N 9°18'12''W	5	Steep rock that continues into sublittoral boulders. The average effective depth of the north shore is 12m. At this shore, there are slow currents in the inflow with speeds of 0.02-2ms^{-1}. Water in the North Basin originates from the South Basin of Lough Hyne.
2 Eastern Shore	51°30'14.6"N 9°17'54.6" W	5	The shore comprises of steep rocks sloping with an angle of 45º and with some boulders.
3 Castle Island	51°30'06'0"N 9°18'02.5" W	6	The shoreline and slope varies greatly on the island. The south side of the island has vertical rock and steep intertidal rock ledges. The north side of the island has moderately (c. 45º) sloping rocks which continue into boulders and mud in the subtidal.
4 Southern Shore	51°29'57''N, 9°17'53'W	7	The slope of the shoreline varies from gentle to near vertical on the South shore of Lough Hyne. In the subtidal the slope is generally gentle. The substrate is composed of gravel and boulders before giving away to mud. The shore experiences powerful currents with speeds of 6-8ms$^{-1.}$
5 Western Shore	51°30'02''N 9°18'22''W	7-8	Steep rock in the intertidal, gently sloped rocks and small stones below low water in the subtidal. At points, it is heavily shaded by overhanging trees. Inflowing water has been recorded to have speeds of 4-6 ms^{-1} as it moves from the South Basin towards the North Basin. The Western Basin contains the deepest point (48m) within Lough Hyne.
6 Barloge	51°29'37.3"N 9°17'45.4''W	6-7	Barloge bay is small and separated by a promontory. The shoreline consists of steeply sloping rock, stones and gravel.
7 Tranabo	51°29'52.7"N 9°17'07"W		The shore is a wide south facing bay connects to Lough Hyne at high water. It takes just under a day for the bay to flush.
8 Tralispean	51°29'54.4"N 9°15'54.1"W	7	Tralispean is a small sheltered bay with slate and gravel as sediment. On the shore there is substantial freshwater runoff across the beach.
9 Tragumna	51°29'54.4"N 9°15'54.1"W	7	Adjacent to Tralispean, it is a small, sandy south/south-west facing beach. Tragumna's shoreline is gently sloping with steep irregular boulders.
10 ToeHead Bay	51°29'49.3"N 9°14'08.2"W	7	The shore is a wide south facing beach with extensive irregular rocky outcrops and boulders.

3.2 Sampling

Patella vulgata were collected from each site 29/06/2015-04/07/2015 (n=30 per site). The sampling period was chosen to coincide with known seasonal peaks of macroparasites of *P. vulgata* (Prinz *et al.,* 2010b). Three limpets were collected from each of ten 0.25m^2 quadrats. Quadrats were haphazardly placed at low shore. In each quadrat, the number of *P. vulgata,* the total number of all *Patella* spp. and percentage cover of macroalgal species *Ascophyllum nodosum*, *Fucus* spp. and *Ulva* spp. were recorded. Limpets found in rock pools, on vertical rock faces and measuring under <10mm in shell length were recorded but not sampled. In total, 300 *P. vulgata* were screened. Salinity was measured at each site with a handheld refractometer (Atago ATC S/Mill-E).

3.2.3 Processing

Patella vulgata were processed immediately after collection. *Patella vulgata* were processed and sexed as specified in Chapter 2, Section 2.2.3, page 43.

3.2.4 Statistical analysis

The factors were Site (fixed, orthogonal, 10 levels) and marine reserve (fixed, nested in location, 2 levels). Analysis of prevalence and median intensity was done by following the approach of recommended by Rozsa *et al.,* (2000), carried out in R studio version 1.0.136 (R Core Team 2015) and detailed further in Chapter 2 Section 5.2 page 44. Differences in prevalence between locations and gonadal stages were tested with a Pearson's chi square with Yates continuity correction. If expected values were too low (<5) for a Pearson's chi square, a Fisher's Exact test was used. Intensities were compared with a non-parametric Kruskal Wallis test if transformation failed to

normalise distributions. Normality of distributions was assessed first visually and then formally by the Shapiro–Wilk test. Variation among locations in host characteristics, namely size, weight, and density, were tested with a one-way analysis of variance (ANOVA). Significant results were investigated with Tukey's Honest Significant Differences (Tukey HSD) or pairwise comparisons following an ANOVA or Kruskal Wallis test. Host weight and shell heights were log transformed, while parasite counts and host density were square root transformed. Parametric tests, Pearson correlations, ANOVAs and Post-hoc tests were carried out in R studio with the packages stats and car (R Core Team, 2017). Statistical significance was considered at $P<0.05$.

3.2.5 Parasite identification

Parasite identification and terminology follows that of that of previous specification in Chapter 2, Section 2.2.3, page 43 & Section 2.2.4 page 44.

3.3 RESULTS

3.3.1 Host morphometrics

The mean shell height of sampled limpets was 17.24 (±3.69, SD) mm. The largest limpets were found in Toe Head Bay and Lough Hyne East (See Table 3), while the smallest individuals were found in Barloge ($F=10.92$, $df=9$, $P<0.001$). The mean density of *P. vulgata* for all sites combined was 48 (±43, SD) gastropods per m^2 sampled quadrats. Density ranged from 18 to 96 limpets per m^2. Highest and lowest densities of limpets were both found in the reserve, on the south (Tukey HSD, $P<0.001$) and north shore, respectively (Tukey HSD, $P<0.001$). The majority of limpets, 229 of the 300 processed, had undifferientated gonads (Fig. 2). Gonadal

stages significantly differed between sites (χ^2=53.10, df=18, P<0.001) with the highest proportion of undifferientated gonads found at Toe Head Bay (Fig. 2).

Table 3 *Patella vulgata* population characteristics (n=30 per site) and site salinity in June 2015 in the form of $\bar{x}$ (±SD).

Site	Host Density per m^2	Shell height mm	Shell width mm	Foot length mm	Whole Weight g	Tissue Weight g	Salinity
1 Lough Hyne North	18 (±5)	16.84 (±3.87)	40.57 (±6.88)	22.65 (±4.60)	13.30 (±6.83)	4.76 (±2.13)	32 (±5.25)
2 Lough Hyne East	36 (±15)	19.94 (±3.20)	46.27 (±6.66)	24.93 (±2.52)	21.25 (±6.56)	8.10 (±5.16)	34 (±1.41)
3 Castle Island	38 (±16)	17.86 (±2.99)	44.55 (±6.53)	24.07 (±3.91)	16.89 (±7.96)	5.75 (±2.51)	32 (±4.97)
4 Lough Hyne South	87 (±31)	16.89 (±3.40)	44.09 (±5.00)	24.14 (±3.80)	15.65 (±6.18)	5.55 (±1.94)	35 (±0.00)
5 Lough Hyne West	31 (±47)	16.32 (±3.15)	45.50 (±5.20)	24.33 (±4.45)	16.94 (±7.34)	6.10 (±2.25)	34 (±1.08)
6 Barloge	20 (±4)	14.83 (±3.25)	39.79 (±3.62)	21.81 (±2.29)	10.74 (±3.64)	3.81 (±1.39)	35 (±0.82)
7 Tranabo	27 (±9)	17.24 (±2.68)	44.00 (±4.42)	26.56 (±3.95)	17.63 (±6.29)	9.45 (±15.65)	28 (±3.68)
8 Tralispean	65 (±15)	19.19 (±3.00)	43.46 (±4.35)	25.44 (±5.67)	15.79 (±7.00)	6.87 (±2.54)	35 (±0.00)
9 Tragumna	96 (±81)	14.68 (±3.95)	35.03 (±5.41)	21.70 (±7.63)	8.27 (±3.49)	3.24 (±3.17)	34 (±1.41)
10 Toe Head Bay	60 (±32)	18.15 (±4.09)	40.97 (±6.13)	23.51 (±4.56)	14.10 (±8.18)	5.22 (±2.92)	35 (±0.00)

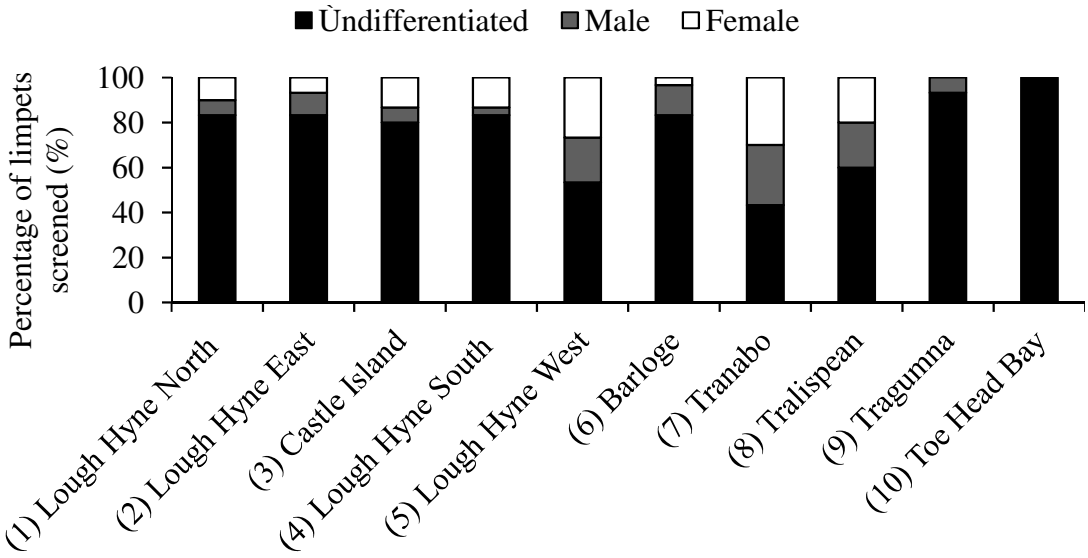

Fig. 2 Sex of the protandrous *Patella vulgata* sampled at each site on the southwest coast of Ireland June 2015.

3.2 Parasite taxa

Of the 300 limpets examined, 52% harboured one or more parasite taxa from four phyla: Annelida, Arthropoda, Ciliophora, or Platyhelminthes. Trematodes were the most prevalent taxa with a total prevalence of 49%. All trematode infections were in the form of metacercariae; an encysted life stage which, upon consumption by a vertebrate, metamorphoses into an adult worm. Trematodes were followed in abundance by ciliates (27%), a cestode *Ophryocotyle insignis* (3%), mites (3%), and a burrowing polychaete *Polydora ciliata* (2%). Five parasitic taxa were recorded within and outside the reserve (Fig. 3). No increase in parasite diversity or intensity of infection was observed within the marine reserve relative to shores outside (Figs 3(b) & 4 (a)) and (Table 4).

Echinostephilla patellae was found in 48% of limpets and occurred at every site sampled except the south shore of Lough Hyne. *Echinostephilla patellae* prevalence and intensity ranged from 7-93% and 1-223 metacercariae per infected limpet (Fig. 3). *Echinostephilla patellae* prevalence significantly differed between sites (χ^2=114.82, df=9, P<0.001) but not between protected and unprotected zones (χ^2 =2.81, df =1, P =0.09) (see Fig 4). High prevalences of *E. patellae* were found in Lough Hyne North (93%) and Toe Head Bay (93%) (Table 4). Low prevalences of *E. patellae* were found in Lough Hyne West (7%). The parasite species was absent from limpets sampled from Lough Hyne South (Table 4). Highest intensities of *E. patellae* were found in Barloge with 223 metacercariae found in one *P. vulgata* (Fig 4 (a)). When grouped by the Ballainte scale, significantly higher intensities of *E. patellae* were observed in sheltered shores (χ^2 =57.74, df=2, P<0.00) (Fig 4(b)).

The remaining trematode infections (1%) were *Gymnophallus* spp., from the family Gymnophallidae. *Gymnophallus* spp. occurred only outside of the reserve in Tragumna. Prevalence of ciliates, most likely the species *Leiotrocha patellae* significantly differed between sites (χ^2=18.01, df=9, P<0.05). Highest prevalence of *L. patellae* was found in Castle Island (43%) and lowest in North Shore (7%). The prevalence of the cestode *O. insignis* varied from 7-58% and 2-114 cysts per limpet found on Toe Head Bay and the North and South shores of Lough Hyne. The polychaete, *Polydora ciliata*, occurred within the reserve on the West shore of Lough Hyne and in Barloge (Fig 2 (b)). *Polydora ciliata* was found in the shells of 3% of limpets at both sites.

Table 4. Median intensity and range of infection of helminth parasites of *Patella vulgata* sampled at each site (n=30)

	Site	*Echinostephilla patellae*	*Gymnophallus* spp.	*Ophryocotyle insignis*
1	Lough Hyne North	30 (6-158)	0	58 (28-87)
2	Lough Hyne East	46 (3-213)	0	0
3	Castle Island	16 (1-192)	0	0
4	Lough Hyne South	0	0	36 (4-114)
5	Lough Hyne West	2 (1-2)	0	0
6	Barloge	18 (1-223)	0	0
7	Tranabo	4 (1-7)	0	0
8	Tralispean	16.5 (2-60)	0	0
9	Tragumna	24 (3-67)	3 (1-67)	0
10	Toe Head Bay	22 (2-130)	0	7 (2-23)

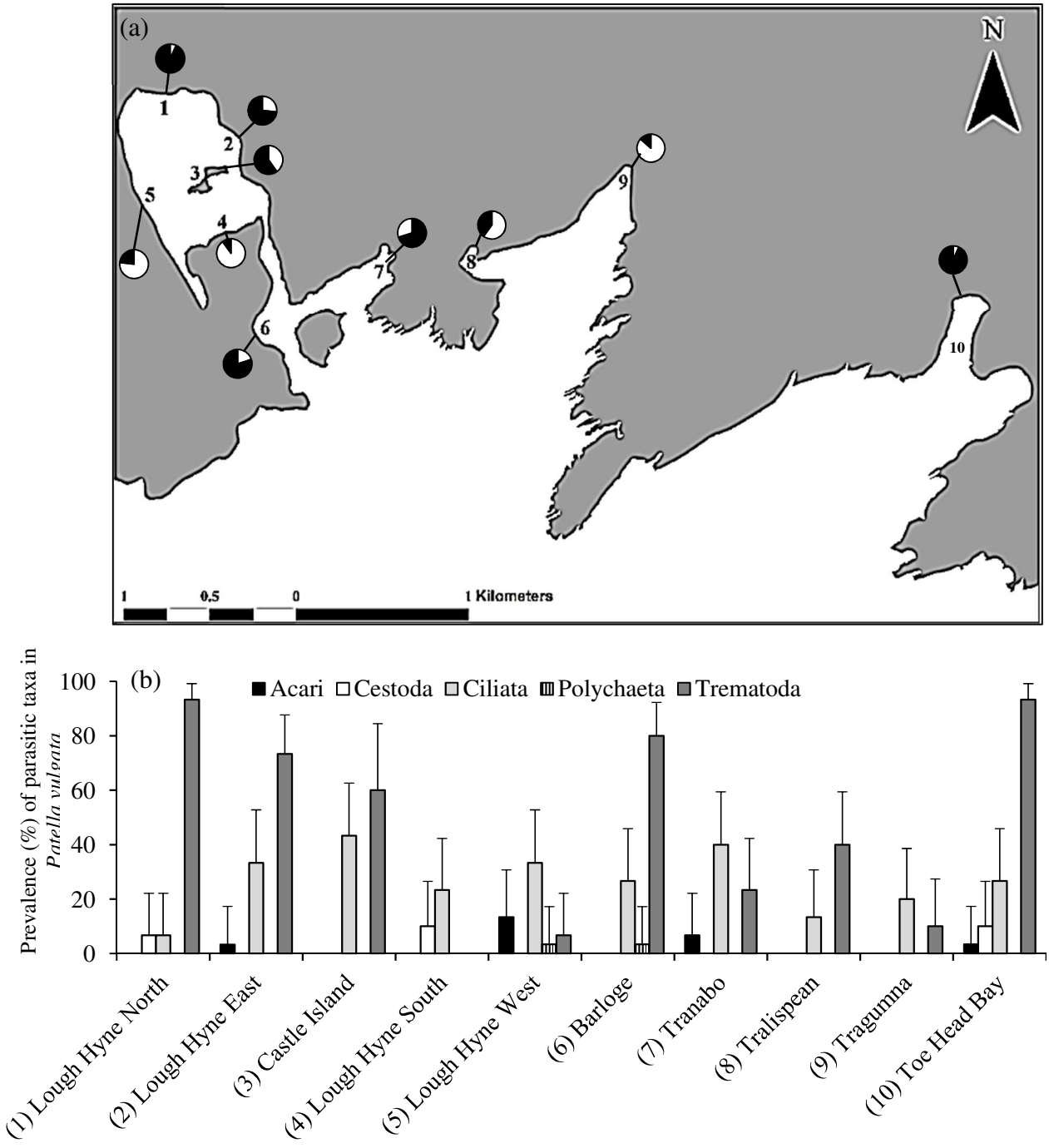

Fig. 3. (a). Map of study sites; 1 Lough Hyne North, 2 Lough Hyne East, 3 Castle Island, 4 Lough Hyne South, 5 Lough Hyne West, 6 Barloge, 7 Tranabo, 8 Tralispean, 9 Tragumna, 10 Toe Head. Pie charts show the proportion of the 30 *P. vulgata* harbouring macroparasite taxa (black) and not parasitised (white). (b) Prevalence of parasitic taxa found in *Patella vulgata* sampled. Error bars are upper 95% Clopper-Pearson confidence intervals. Map adapted from Environmental Protection Agency Water Quality shapefile.

3.3.3 Parasite and host dynamics

Echinostephilla patellae had an overall prevalence of 48% and was the dominant parasite in this study, so further analysis focused on this species. *Echinostephilla patellae* prevalence significantly differed between gonadal stages (χ^2=53.1, df=2, P<0.001). A higher prevalence of *E. patellae* was found in *P. vulgata* with undifferientated gonads . The largest proportion of undifferentiated gonads was found on Toehead Bay (Fig.2). However, *E. patellae* intensity did not differ between gonadal stages (F=1.008, df=2, P>0.05). *Echinostephilla patellae* prevalence and intensity were negatively and significantly correlated with *Patella* spp. density (r=-0.27, df=297, P<0.0001) and (r=-0.22, df=297, P<0.001) repsectively. *Echinostephilla patellae* prevalence and intensity correlated with limpet size (r=0.12, df=298, P<0.05) and (r=0.13, df=298, P<0.05). *Echinostephilla patellae* prevalence did not significantly differ with other host morphometrics, namely, shell width, whole organism or wet tissue weight (Table 2).

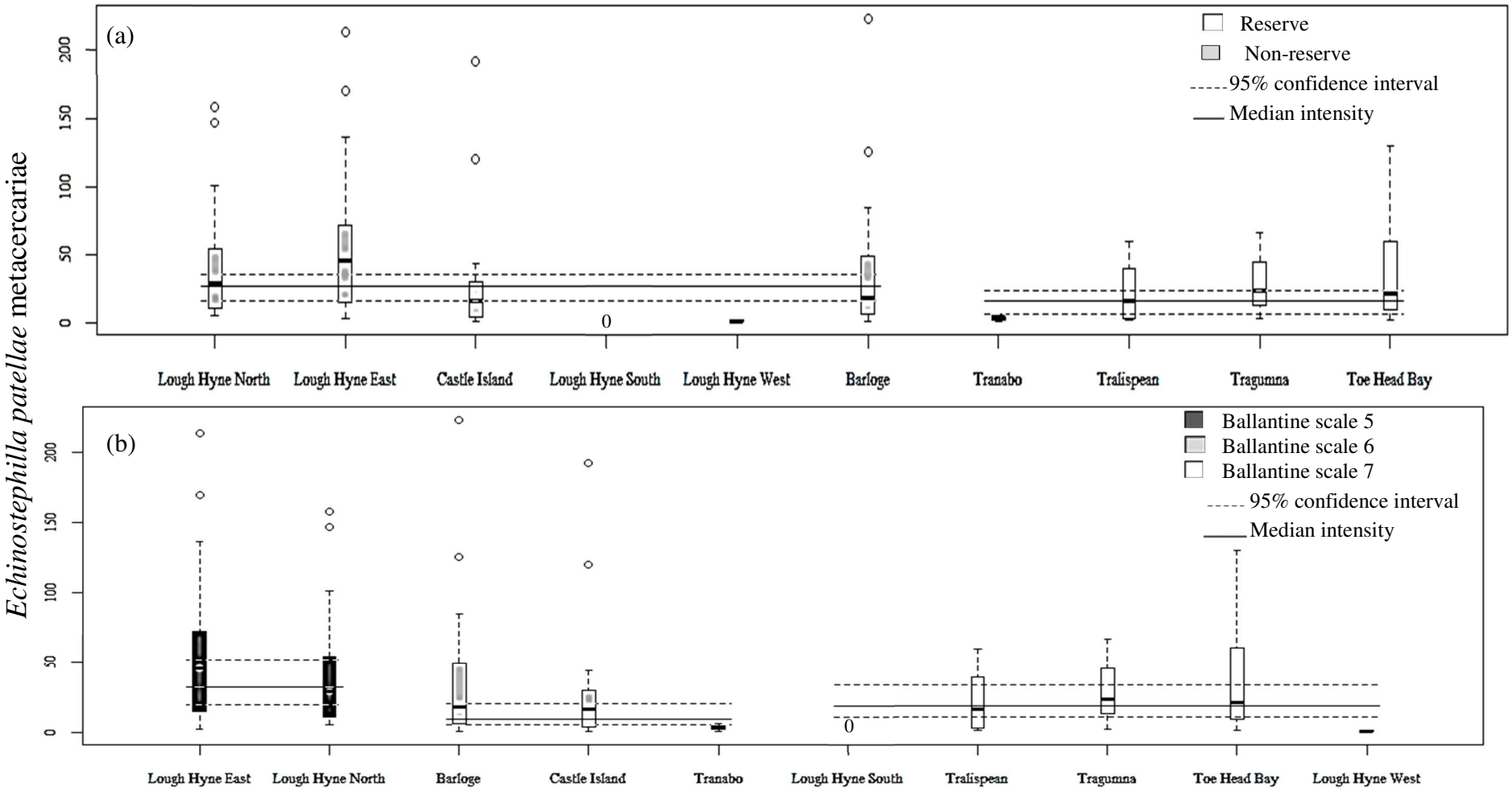

Fig. 4 The median intensity of *Echinostephilla patellae* metacercariae per sampling site and averaged (a) over protected and unprotected zones or (b) the sampling sites' Ballantine scale values.

3.4 DISCUSSION

In the current study, parasite prevalence, intensity and richness in *P. vulgata* did not vary in relation to the level of legal protection of a site. Consequently, other factors must determine the distributions recorded. Determining factors in macroparasites recorded are likely to be local site factors such as water movement and host population's characteristics (Hechinger *et al.,* 2007, Byers *et al.,* 2008).

Coastal configuration is known to play a role in the retention of water in bays. It also affects the structure of benthic invertebrate communities and therefore is likely to affect the parasite communities recorded in this study. In the water column, cercariae may be functionally similar to benthic lecithotrophic larvae (Crewe, 1951, Kollien, 1996, Zimmer *et al.,* 2009). Cercariae recorded in this study are similarly sized (<1mm) to benthic invertebrate larvae of previous work in the area (Crewe, 1951, Kollien, 1996, Jessopp, 2007, Jessopp & McAllen, 2008). Cercariae are also soft bodied, free living, and short lived (<24 hours) (Kollien, 1996, Pietrock & Marcogliese, 2003). This stage is sensitive to a range of environmental conditions and has a suite of behaviours which increases the probability of contacting an appropriate second intermediate host (Pietrock & Marcogliese, 2003, Sukhdeo & Sukhdeo 2004, Thieltges, 2006, Zimmer *et al.,* 2009).

Evidence for the role of configuration and degree of trematode infection is illustrated through *E. patellae*. Previous work on the role of wave exposure regarding the prevalence of *E. patellae* cercarial infection is contradictory with sheltered (Thomas, 1965) and exposed shores (James 1968) in British waters. In the present study, higher prevalences of *E. patellae* tended to be recorded on shores with slower currents, gentle slopes and long water retention times namely, Barloge, Castle Island, the North and East shore in Lough Hyne and Toe Head Bay outside of the reserve. However, this pattern was not statistically significant.

The highest intensity of *E. patellae* was recorded in Barloge. This is likely due to slow moving water and low host densities. Under laboratory conditions, *Patella* spp. infected by *E. patellae*, release a pulse of cercariae, up to 500, after immersion (Crewe, 1951). At the beginning of a flood tide, when water in Barloge is practically still (Bassindale *et al.,* 1958), so a mass release of cercariae may occur in an area of low host density. Potentially, multiple cercariae could reach and infect the limited number of *Patella* spp. in Barloge with minimal interference by current. Low prevalence of *E. patellae* was recorded on shores with steep slopes and exposure to faster water currents. Prevalence of *E. patellae* was low on Western shore and absent on the Southern shore of Lough Hyne. The absence of *E. patellae* on the Southern shore is likely due to turbulent currents at the "rapids". In this channel, significant mortality of invertebrate larvae occurs (Bassindale *et al.,* 1948, Jessopp, 2007). The turbulence probably has a similar effect on *E. patellae* cercariae.

In sheltered shores cercariae may persist for longer in the water column. This increases the probability of cercariae finding a suitable host and decreases the probability of cercariae being dislodged during the infection process. Lough Hyne is an extremely sheltered habitat with limited tidal exchange between outer waters due to its unusual coastal configuration. Evidence for the degree of wave exposure is provided by Swedish rocky shores where increasing wave exposure coincided with low trematode prevalence in *Littorina saxatilis* (Granovitch *et al.,* 2004).

Echinostephilla patellae prevalence or intensity were not correlated with host morphometrics observed in this study. Greater prevalence of *E. patellae* metacercariae was recorded in *P. vulgata* with undifferentiated gonads. However, no significant difference in *E. patellae* intensities was recorded between gonadal stages. Thus, higher prevalence in undifferentiated gonads is likely due to the large proportion of *P. vulgata* with undifferentiated gonads in populations sampled, rather than a preference of *E. patellae*. The proportions of gonadal stages in populations agree with

histological work on a *P. vulgata* population on a moderately exposed shore. It was found that most *P. vulgata* sampled had undifferentiated gonads in June and matured in December (McCarthy *et al.,* 2008).

The composition of the parasite community and its variation over small spatial scales agreed with previous records for Ireland (Copeland *et al.,* 1987, Prinz *et al.,* 2010a) and the UK (Crewe, 1951, Thomas, 1965). Parasite communities in *P. vulgata,* like other molluscan hosts, were dominated by trematodes, while other metazoans, such as the cestode *O. insignis*, were less common (Sousa, 1994, Poulin, 2001, Prinz *et al.,* 2010a). Moreover, species richness and occurrence were similar to *P. vulgata* parasite communities recorded a year previously (Chapter 2) for the Western shore and Tralispean.

There was a notable absence of trematode life stages preceding metacercariae, the cercariae, and the taxon "Cercaria B" (Crewe, 1951). These absences may be due to sampling the low shore at sites, which was chosen in order for sites outside the Lough to be comparative due to the restricted tidal range in Lough Hyne. High levels of cercarial infections have been recorded at the mid tide level of rocky shores in previous studies (Thomas, 1965, Prinz *et al.,* 2010b) due to the foraging preference of parasites' avian hosts. For *E. patellae* and for the cestode *O. insignis* the oystercatcher, *Haematopus ostralegus* is a recorded final host, (Burt, 1962, Kollien, 1996), and for *Gymnophallus* spp. gulls, *Larus* spp., are considered likely final hosts (Crewe, 1951). While the mobility and behaviour of avian hosts play a major role in the distribution of the species (Hechinger *et al.,* 2005, Feis *et al.,* 2015), they were not surveyed during the study. Local site factors favouring gull final hosts have been found to the major determinant of trematode prevalence in *Littorina* spp. in New England (Hechinger *et al.,* 2005, Feis *et al.,* 2015). The small spatial scale (<10km) of the present study means that all sites sampled were within the dispersal range of final avian hosts. However,

only low numbers of *H. ostralegus* have been observed in the reserve (Kelly, T pers. comm., 2015).

This study found that *P. vulgata* populations within the reserve do not function as a parasite reservoir per the definition provided by Haydon *et al.,* (2002). No effect of the reserve was detected in relation to parasite or host populations. Turbulent currents at the "rapids" makes it likely that cercariae from inside the reserve will not transfer or "spillover" into *P. vulgata* populations outside of the reserve. Hosts within a reserve do not automatically become resistant to infection or act as a reservoir for their parasites. Caution is urged when drawing the conclusion that a marine reserve could not act as a reservoir or amplify parasite dynamics. Lough Hyne, although well studied, may not be representative of other marine reserves due to its sheltered nature and limited tidal exchange with outer waters. In particular, the limited tidal range may have negative implications for the available space and time spent by potential avian hosts on the shores foraging. It was designated because of its biological diversity and varied habitats contained in a small area (Bell & Barnes*.,* 2002). However, recent MPA designations have specific criteria e.g. the restoration of commercial species or habitat. Proposed networks of MPAs will have a high degree of connectivity due to increased water movement and strategic locations. These MPAs may have greater potential to facilitate parasite transmission and act as reservoirs (Fenberg *et al.,* 2012).

Multiple factors drive disease or parasite development. The ecological consequences of recorded taxa merit further work due to the ecological relevance of *P. vulgata* and the ubiquity of parasite taxa. In general, this is also true of parasite taxa of other invertebrate species. It is necessary to consider non-commercial species or neglected parasite taxa in MPAs. This is in order to avoid a panglossian view of MPAs and to ensure their conservation objectives are achieved.

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CHAPTER 4: A case study on macroparasite infections in the intertidal invertebrates, the green shore crab (*Carcinus maenas*) and ragworm (*Hediste diversicolor*) in nutrient enriched estuaries.

ABSTRACT

Eutrophication is a widespread stressor in the marine environment. Eutrophication may encourage parasite taxa by increasing the density of gastropod hosts and enhancing the production of free living infectious stages via increased host survival. This study examined the occurrence of macroparasites in the intertidal invertebrates, *Carcinus maenas* and *Hediste diversicolor,* from three estuaries of differing nutrient classifications. *Carcinus maenas* and *H. diversicolor* are abundant in estuaries, have differing ecological roles, wide geographic distributions, and previously have been used in biomonitoring programmes. Both invertebrate taxa may be valuable host: parasite models to evaluate the response of parasite communities to eutrophic conditions. Nutrient classifications were provided by the Water Framework Directive and characterised by the parameters, ammonia, ortho-P, total oxidised nitrogen, total phosphorus, total nitrogen, salinity and temperature. To account for potential vertebrate host abundance, bird communities were surveyed once a month, at fixed points within the three selected estuaries. From the 1,002 invertebrates screened, parasitic taxa from the classes Trematoda, Cestoda, Copepoda, Nematoda and Acari were recorded. Digenean trematodes were the most common parasite infection of *C. maenas* and *H. diversicolor*. *Carcinus maenas* harboured few taxa but was heavily infected by the trematode metacercariae of *Microphallus* spp. This study found a relationship between eutrophication levels and trematode distribution in estuaries. Higher abundances of potential avian hosts and digenean trematodes were recorded in the enriched estuary. Given the predicted increases in eutrophication, and the global

distribution of invertebrate hosts screened, this study has broad ecological significance.

4.1 INTRODUCTION

Estuaries are some of the most productive, diverse, and economically important ecosystems on earth (Hobbie, 2000). Approximately 75% of the world's population are dependent on estuarine and coastal watersheds (Vitousek *et al.,* 1997, Small & Nicholls, 2003). In Ireland, over half of the population resides within 10km of the coastline (Stapleton *et al.,* 2000). Globally, high levels of anthropogenic impacts such as urbanisation, industrialisation, commercial fishing, and intensification of agriculture, have resulted in estuaries becoming some of the most impacted ecosystems (Jackson *et al.,* 2001, Lotze *et al.,* 2006, Airoldi & Beck, 2007). Nitrogen from terrestrial sources, agricultural runoff and sewage discharge, are all considered causal factors for eutrophication in marine ecosystems (Howarth & Marino, 2006). Eutrophication is a chronic widespread stress on marine ecosystems on a global scale (Smith, 2003) and a national scale as over half of coastal waters and estuaries in Ireland are affected by the stress (O'Boyle *et al.,* 2010, Hartnett *et al.,* 2011). The extent and severity of eutrophication will likely increase in response to growing human populations (Smith, 2003, Lotze *et al.,* 2006).

Eutrophication is broadly speculated to encourage parasitic infection directly, by providing nutrients to opportunistic parasite taxa to assimilate and indirectly by changing ecological communities' composition (Lafferty, 1997, McKenzie & Townsend, 2007, Budria, 2017). The facilitation maybe be threshold dependant as low to moderate eutrophication may favour parasite taxa able to infect multiple hosts and those with complex lifecycles that involve benthic invertebrates or fish (Esch, 1971, Galli *et al.,* 2001, Zander & Reimer, 2002, Johnson & Carpenter, 2008). The survival of infected hosts may increase with the availability of resources and the carrying capacity of potential Low to moderate eutrophication can affect hosts (Lafferty & Holt, 2003, Smith & Schindler, 2009). However, severe eutrophication may inhibit parasite infection due to the resulting hypoxia, mass invertebrate

mortalities, blooms of harmful algae and macroalgae (Hull, `1987, Raffaelli, 1999, Zander *et al.,* 2002, Howarth & Marino, 2006). Macroalgal blooms could impair the survival and movement of free living parasite life stages and oxygen deficiency could decrease host abundance and survival (Dušek *et al.,* 1998, Zander & Reimer, 2002, Pietrock & Marcogliese, 2003).

Parasitic trematode life cycles contain free living infective stages which are sensitive to a range of environmental conditions (Pietrock & Marcogliese, 2003). Eutrophic conditions in freshwater ecosystems encouraged trematode infection by increasing the abundance of intermediate hosts and the number of cercariae produced by primary intermediate hosts (Johnson *et al.,* 2007, Johnson & Carpenter, 2008). In the intertidal zone of estuaries and coastal shores, trematodes dominate invertebrate macroparasite communities (Dobson *et al.,* 2008, Kuris *et al.,* 2008, Fermer *et al.,* 2011). In estuarine ecosystems, trematodes collectively, can reach a substantial biomass. In three Californian estuaries, trematode biomass was equivalent to that of birds, fishes, burrowing shrimps, and polychaetes (Kuris *et al.,* 2008). Consequently, evaluating the effect of eutrophication on parasites, such as trematodes, in ecosystems where they are abundant, is crucial. This study, aimed to investigate the potential of eutrophication to encourage macroparasite infection of two invertebrate taxa in estuaries. It was hypothesised that enriched estuaries would experience greater richness and intensity of parasitic infection.

The two invertebrate taxa chosen to be screened in estuaries of differing levels of enrichment were the green shore crab *Carcinus maenas* and polychaete *Hediste diversicolor.* Both invertebrates are abundant in estuaries, have wide physiological tolerances and Atlantic-wide distributions (Crothers, 1967, Taylor *et al.,* 1973, Scaps, 2002). As a decapod crab, *C. maenas* experience a high degree of parasitic infection from a range of macroparasite taxa (Thieltges *et al.,* 2009, Lyons, 2011). Outside of its native range *C. maenas* is found in Pacific North America, southern Australia,

Tasmania, Japan and South Africa (Crothers, 1967, Carlton & Cohen, 2003). The success of *C. maenas* as an invasive is partly attributed to its escape from native parasites (Torchin *et al.,* 2001, Zetlmeisl *et al.,* 2011). Polychaetes are parasitised by a range of helminth taxa (Margolis, 1971; 1973, Vismann, 1990), but are rarely considered in parasitological studies (Margolis, 1971, 1973, Peoples *et al.,* 2012). *Hediste diversicolor* play a role in the bioturbation of sediment, are a common prey of birds and have potential economic value as a bait fishery (Scaps, 2002, Nybakken & Bertness, 2005, Waston *et al.,* 2016).

Bird communities were also surveyed as final hosts are known to play a key role in the distribution of macroparasites (Hechinger & Lafferty, 2005, Levakin *et al.,* 2013, Feis *et al.,* 2015).

4.2. MATERIALS AND METHODS

4.2.1 Study Sites

Three estuaries were sampled on the southwest coast of Ireland; Clonakilty (51°36'24.3"N 8°52'37.5"W), Oysterhaven (51°42'20.8"N 8°27'37.5"W) and Youghal (51°97’ 862” N, 007°85’ 844” W) (Fig. 1) Clonakilty Bay is a small estuary (approx. 272 ha) with mud and sand flats. It is a proposed Special Protection Area (SPA), a proposed Natural Heritage Area (NHA) and a candidate Special Area of Conservation (SAC). Clonakilty estuary has transient algal mats within the bay and the town sewage inflow is situated at the head of the estuary. Youghal is a moderately-sized (approx. 729 ha), sheltered south-facing estuary. Youghal is an SPA into which the town sewerage system discharges untreated effluent to the harbour via 3 sewage outfalls. Oysterhaven is a small (approx. 100 ha) south facing estuary. Oysterhaven estuary is not an SPA but is adjacent to the Sovereign Islands SPA. The nutrient statuses of Irish estuaries were assigned by the Environmental Protection Agency (EPA) according the

Water Framework Directive (WFD). Estuaries' nutrient statuses were evaluated by the analysis of water parameters ammonia (NH_3), ortho-phosphate (MRP), total nitrogen (TN), total oxidised nitrogen (TON), total phosphorus (TP) as mgL^{-1}, temperature and salinity. Clonakilty was classified as eutrophic by the EPA according to the classifications of WFD This was due to elevated nutrient concentrations and accelerated growth of macroalgae. Youghal and Oysterhaven were classified as "moderate" and "unpolluted" water quality according the WFD (O'Boyle *et al.,* 2010). "Eutrophic" status is awarded when threshold values for nutrients, macroalgal growth and an "undesirable disturbance" are exceeded. "Moderate" status is awarded when two criteria were breached and "unpolluted" status when no criteria were exceeded.

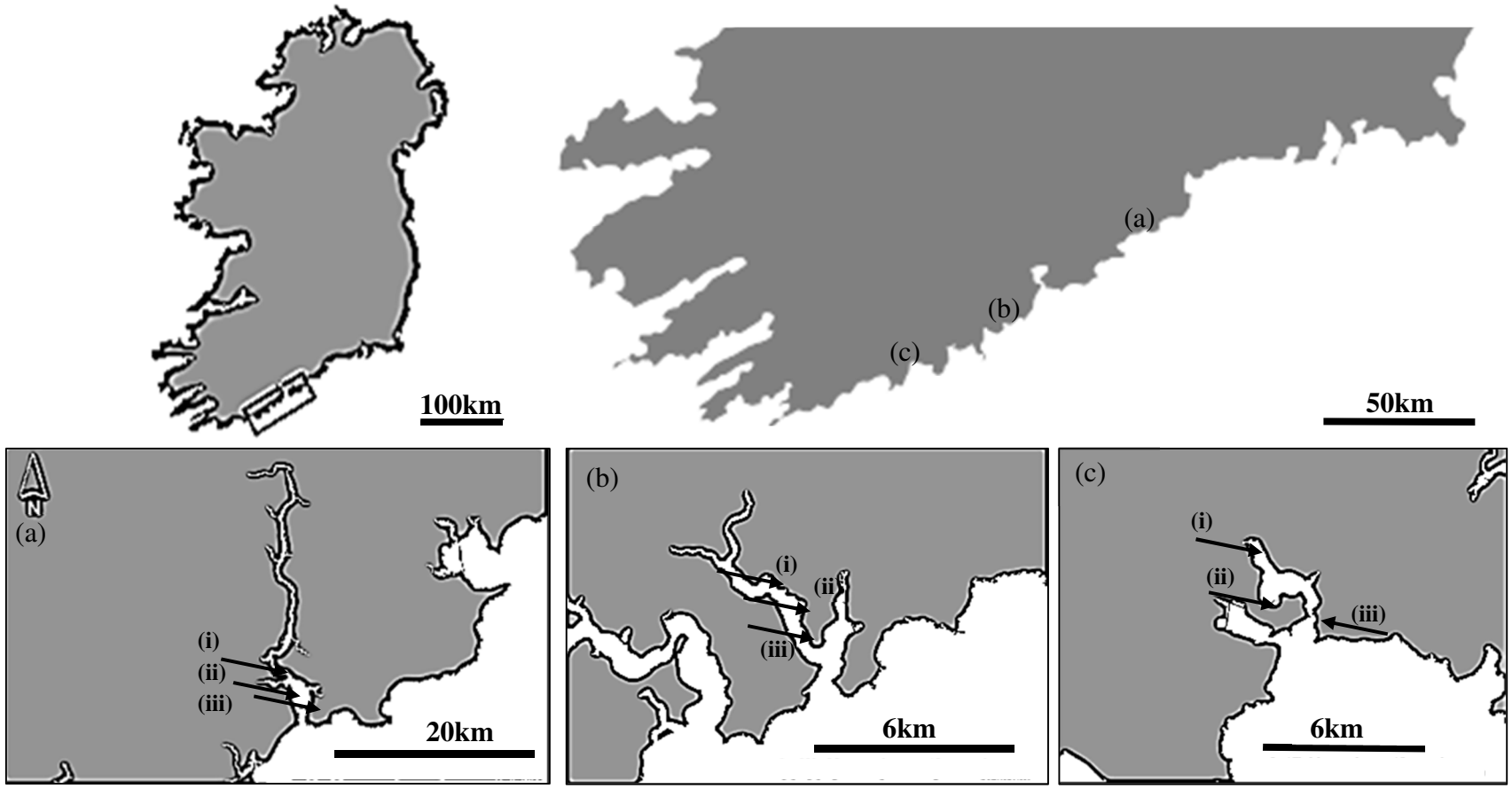

Fig. 1. Study sites (a) Youghal estuary (b) Oysterhaven estuary (51°42'51.8"N 8°28'29.0"W), middle (51°42'29.5"N 8°27'32.2"W) and outer (51°42'04.5"N 8°27'30.4"W) points and (c) Clonakilty estuary. (i) Inner, (ii) middle and (ii) outer sampling points with sites designated with an arrow. Adapted from Environmental Protection Agency Water Quality shapefile.

4.2.2 Sampling

4.2.2.1 Host samples

Carcinus maenas and *H. diversicolor* were sampled at three fixed points within each estuary in June, July and August 2015 (Fig. 1). Sampling points were at the inner, middle and outer zones of the estuary where *C. maenas* was abundant to account for spatial variation in salinity. In Clonakilty estuary, the inner (51°61'932" N, 008°88'103" W), middle (51°36'264"N, 008°52'454"W), and outer (51°36'10.9"N, 8°51'14.8"W) points in were sampled. The inner (51°97'862" N, 007°85'844" W), middle (51°95'881" N, 007°84'951" W) and outer (51°94'641" N, 007°84'174" W) of Youghal estuary. In Oysterhaven, the inner (51°42'51.8"N 8°28'29.0"W), middle (51°42'29.5"N, 8°27'32.2"W) and outer (51°42'04.5"N, 8°27'30.4"W) points. The inner (51°58'22.6"N, 7°51'31.3"W), middle (51°57'49.9"N, 7°51'03.5"W) and outer (51°56'46.8"N, 7°50'32.9"W) of Youghal estuary. At low tide, *Carcinus maenas* and *Hediste diversicolor* were haphazardly sampled respectively, by hand or from the top 20 cm of sediment until 30 of each were found.

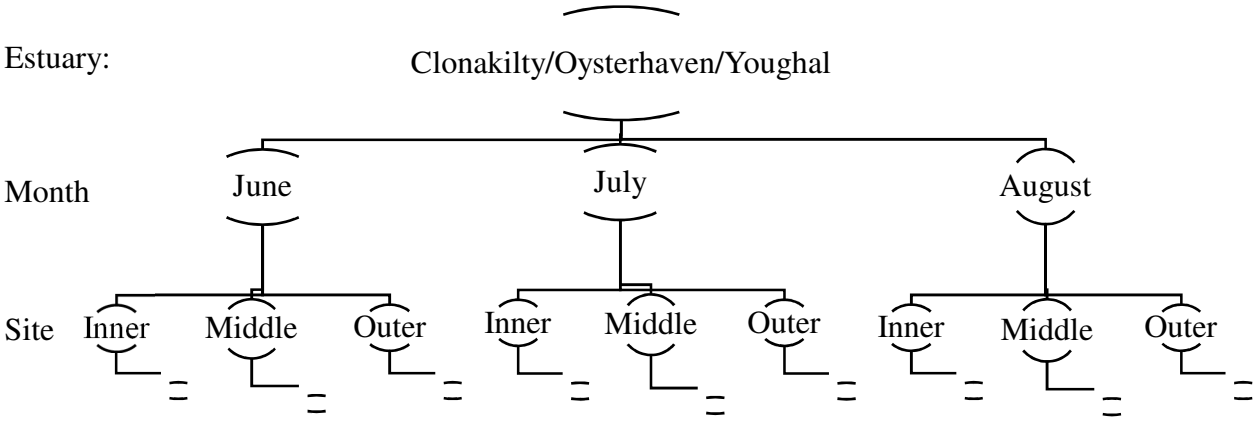

Fig. 2 Schematic diagram of the sampling programme for intertidal invertebrates and water samples for each estuary. Factors are shown on the left.

4.2.2.2 Potential avian host surveys

Each month, point censuses were taken of bird populations at the mid points of estuaries sampled (Fig. 3). Within a week of invertebrate sampling the abundance of all species was recorded at the same locations with survey methodology based on that of Bibby *et al.,* (2000). Surveys were conducted during a flood tide, occurred within a week of invertebrate sampling events and lasted an hour and thirty to forty-three minutes. Ambiant temperature and wind speed were taken. Cloud cover was categorised in oktas as defined by the World Meteorological Organisation (WMO); 0 for clear skies, 1–2 for few clouds, 3–4 for scattered clouds, 5–7 for broken clouds, and 8 for overcast skies.

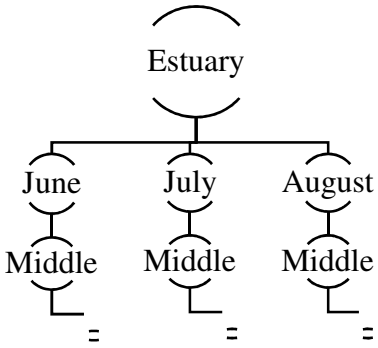

Fig. 3 Schematic diagram of the sampling programme for point censuses of bird populations

4.2.2.3 Water samples

Samples of estuarine water were collected during the flood tide at the three sampling points in each estuary on a monthly basis. At each of these points 500ml of water was collected in opaque plastic containers previously washed once with 10% HCL solution and twice with deionised water. Salinity and temperature of water were measured at the exact location of sampling, prior to the emersion of a container, with a handheld

conductivity meter (Cond 330i/Set 2C20-001). Turbidity was estimated with a secchi tube. For each sample, containers were opened just below the surface (ca. 10cm), in depths of less than 0.5m, away from sediment disturbance and approximately 3 hours after low water. Containers were sealed under water, after at least a minute post immersion, then placed on ice and brought to the laboratory for processing as soon after sampling as possible.

All water analysis was carried out by the accredited Aquatic Services Unit (ASU) laboratory in UCC. The ASU regularly participates in an international laboratory proficiency scheme (Aquacheck) to ensure the quality of the data generated. Water chemistry parameters, Ammonia (mg/L), Ortho-P (mg P/L), Total Oxidised Nitrogen (mg N/L), Total Phosphorus (mg P/L) and Total Nitrogen (mg N/L), were measured using standardised laboratory procedures. Ammonia (Total Ammonia Nitrogen, including both NH_4^+ and NH_3) was analysed with flow injection analysis (FIA) using the supplied method QuikChem Method 10-107-06-3-D. Ortho-P was analysed manually on filtered samples using the Murphy Riley (1962) molybdate/ascorbic acid method. TON was analysed with FIA using the supplied method QuikChem Method 10-107-04-1-C. TP was analysed manually on unfiltered samples using the Murphy Riley (1962) molybdate/ascorbic acid method after sample digestion. TN was analysed with FIA on unfiltered samples using the supplied method QuikChem Method 10-107-04-1-C after sample digestion.

4.2.4 Processing

Invertebrates were frozen after collection (ca.-18°C), as freezing does not affect size of trematodes' metacercariae (Lepitzki *et al.,* 1994). After defrosting and prior to examination invertebrates were weighed, sexed and in the case of *C. maenas,* carapace width was measured. Ten of the thirty *C. maenas* collected were haphazardly subsampled and classified as male or female depending on the number and development of pleopods (Shen, 1935). *Hediste diversicolor* were classed as female with the presence of large eggs (≥200µm), male with white masses in the coelom and undifferentiated with the absence of both (Dales 1950). The visceral cavity of *C. maenas* was excised and examined as trematode metacercariae are concentrated within the area (Torchin *et al.,* 2001, Torchin *et al.,* 2002, Blakeslee *et al.,* 2009). Whole mounts of *H. diversicolor* were examined. All tissue squashes were examined under x40 to x100 magnifications with transmitted light. Parasites were identified based on morphology and descriptions provided by Burt (1962), Crothers (1968), Brock & Lightner (1990), Meyers (1990), Raftos & Cooper (1990), Koie (2000), Pina *et al.,* (2011) and Peoples *et al.,* (2012). Larval trematodes were distinguished according to their colouration, size and if present, acetabula (suckers), excretory systems, stylet and eyespots. For metacercariae, the cyst wall or collar spines, again if present, were also used to distinguish between taxa. Cestode taxa were identified by the lack of an alimentary canal and the presence of the protoscolex (precursor to attachment sucker) and cercomer (caudal appendage). Parasitological terminology and microscopes follow the specifications of Chapter 2 Section 2.2.3 page 43.

4.2.5 Analysis

The water and invertebrate samples were 3 factor analyses. The factors were estuary (fixed, orthogonal, 3 levels), month (fixed, random, 3 levels) and site (random, nested in estuary, 3 levels) (See Fig. 2). The bird point surveys were two factor analyses with

two factors estuary (estuary, fixed, orthogonal, 3 levels) and month (fixed, orthogonal, 3 levels). Analysis was carried out in R studio (R Core Team, 2017).

4.2.5.1 Invertebrate and parasite data analysis

Normality was formally tested by the Shapiro–Wilk test. Homogeneity of variance was then checked with the Cochran test. Prior to analysis, non-normal data were transformed; count data were transformed via square root, proportional data transformed via arcsine and continuous data were transformed via log. If transformation failed to normalise the distribution of data, non-parametric tests were carried out. Host characteristics were compared with a parametric ANOVA test and a post hoc Tukey honest significant difference (Tukey HSD) test. Prevalences between sites, zones, sampling events and sexes were compared with a non-parametric Pearson's Chi-squared test with Yates Correction. Intensity of infection was compared with the non-parametric Kruskal-Wallis rank sum test and a paired Wilcoxon test with Bonferroni correction. Statistical significance was considered at $P<0.05$.

4.2.5.2 Water samples

Physico-chemical parameters were compared with a parametric ANOVA test and a post hoc Tukey HSD test.

4.2.5.3 Potential avian host surveys

Abundance and species richness were the factors considered in the survey. Richness was compared with the Shannon-Wiener diversity index (H). Pearson correlations between the counts of bird taxa listed as final hosts of trematode taxa recorded and trematode intensity were carried out. $N(N-1)/ \Sigma n(n-1)$

4.3. RESULTS

4.3.1 Parasite taxa

In total, five macroparasite taxa were recorded. Trematodes, a cestode, unidentified copepods, nematodes and a halacarid mite were recorded in the 1,002 invertebrates screened (Fig.4). Trematodes dominated the parasitic communities of both host species screened at all sampling events and all sites (Fig. 4). Trematode infections in *C. maenas* were solely metacercariae of a microphallid, most likely *Microphallus primas* (Jägerskiöld, 1908). However, the trematode is referred to as *Microphallus* spp. hereafter as morphologically similar but distinct microphallid species have been recorded in the region in *C. maenas* (Gibson *et al.,* 2005, Leung *et al.,* 2009, Lyons, 2011) and *Pagurus bernhardus* (Linnaeus, 1758) (Lynch *et al.,* 2015). This was similar to the approach of Lynch *et al.,* (2015), in the preliminary survey of *P. bernhardus* (Lynch *et al.,* 2015). *Microphallus* spp. was the most common parasite recorded in the study with 82% of all *C. maenas* infected. *Hediste diversicolor* was parasitised by a range of parasite taxa: trematodes, larval nematodes, cestodes, copepods and mites (Fig. 4a). Trematode infections of *H. diversicolor* consisted of metacercariae of an echinostomatid, likely *Himasthla militaris* (Rudolphi, 1802), and a gymnophallid, likely *Gymnophallus choledochus* (Odhner, 1900). Metacercariae of the echinostomatid trematode (15%) were more common than the gymnophallid (5%). The nematode, *Cucullanus* sp. (Müller, 1777), and an unidentified copepod harboured by *H. diversicolor* were the next most abundant parasite as both had a prevalence of 2%. The cestode, *Ophryocotyle proteus,* Friis, 1870, and halacarid mite were the least common taxa with a prevalence of 0.15%. The site richest in parasitic taxa was the enriched site, Clonakilty with 7 parasitic taxa recorded, and was followed by the Oysterhaven (5), and Youghal estuaries (4).

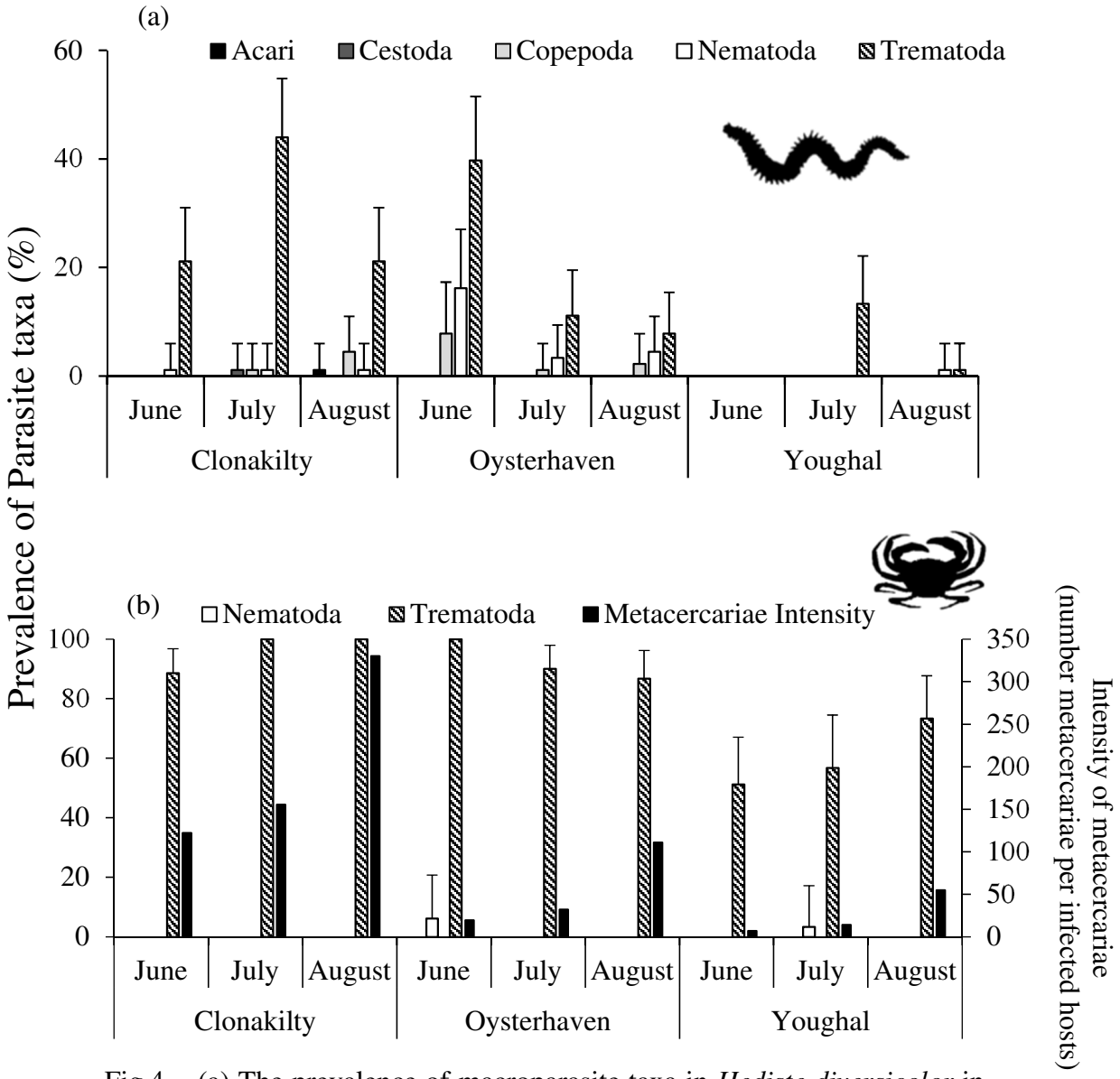

Fig.4 . (a) The prevalence of macroparasite taxa in *Hediste diversicolor* in estuaries per site and sampling event in 2015. (b) The prevalence of trematode *Microphallus* spp. metacercariae taxa in *Carcinus maenas* per site and sampling event in 2015. Error bars for prevalence are upper 95% Clopper Pearson Intervals. Median values of metacercariae intensities are plotted on the secondary axis. Note the differing y-axes between (a) and (b).

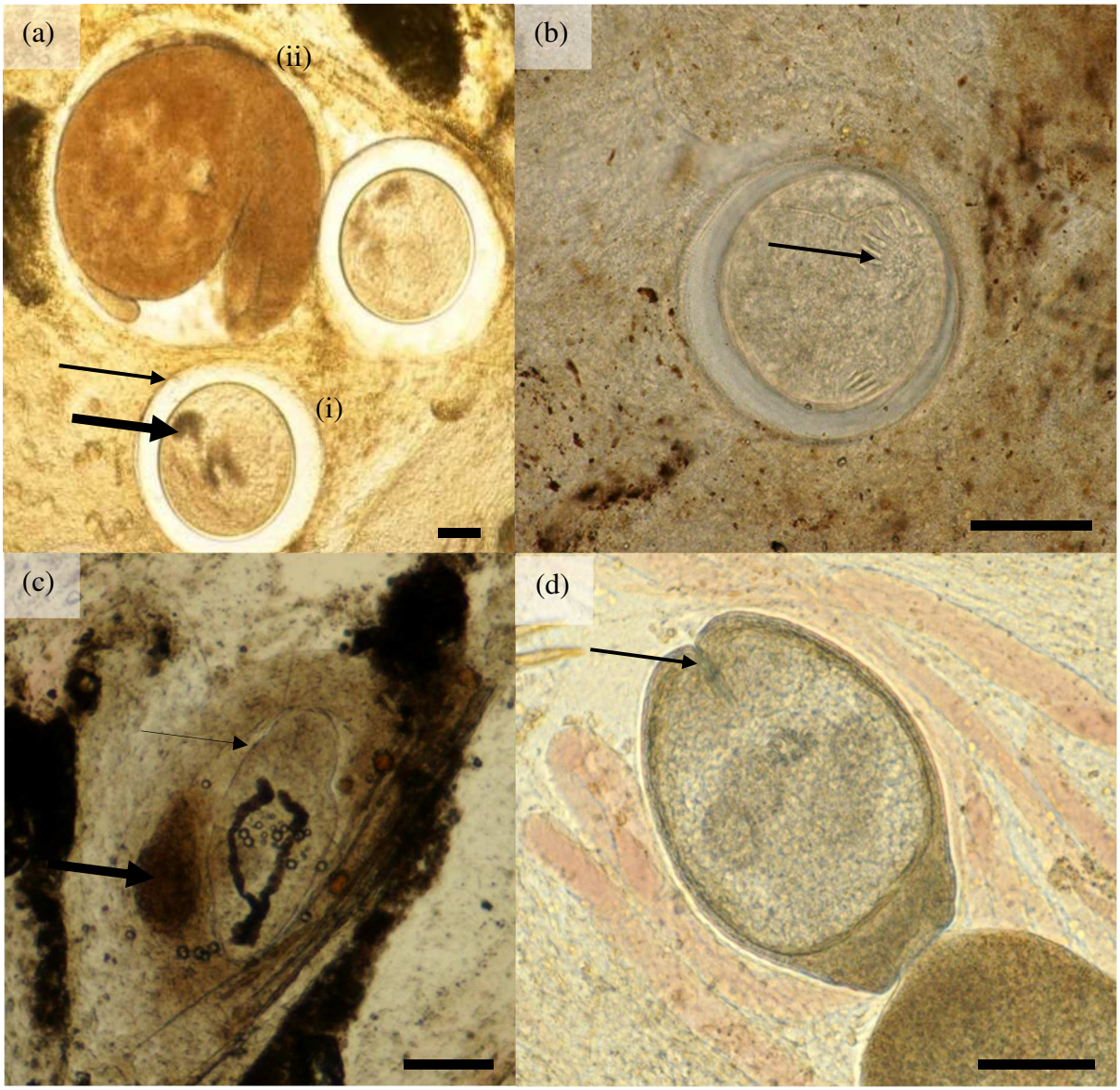

Fig. 5. Photomicrographs of helminth larvae recorded at three estuaries on the southwest coast of Ireland. (a) Microphallid metacercaria in the two developmental stages (i) and (ii). Note the characteristic thick cyst wall (arrow) and dark gonad and vitellarium tissue (thick arrow); (b) Echinostomatid metacercariae with collar spines (arrow) which are characteristic of the family; (c) Gymnophallid metacercariae, note the lack of cyst wall (arrow) and surrounding orange colouration (thick arrow); (d) *Ophryocotyle* sp. cysticeroid with protoscolex (arrow). Microphallid metacercariae were recorded in *Carcinus maenas.* Helminth larvae (b-d) were recorded in *Hediste diversicolor.* Scale bars denote 100µm.

Carcinus maenas

Microphallus spp. prevalence (χ^2=54.31, df=2, P<0.0001) and intensity (χ^2=104.09, df=2, P<0.0001) significantly differed between estuaries. For all sampling events, higher prevalences and intensities of *Microphallus* spp. were recorded in the enriched site, Clonakilty estuary, relative to Oysterhaven and Youghal estuaries (Fig. 4 & 6). The most significant differences in prevalence and intensity occurred between Clonakilty and Youghal estuaries (P<0.0001). Overall, 96%, 92% and 59% of *C. maenas* were infected with *Microphallus* spp. in Clonakilty, Oysterhaven and Youghal estuaries, respectively.

Prevalence of *Microphallus* spp. in *C. maenas* varied within estuaries (see Fig. 4). In Youghal estuary, *Microphallus* spp. was significantly more prevalent in the inner and middle sites relative to the outer site (χ^2=33.20, df=2, P<0.0001). Intensity of *Microphallus* spp. within estuaries differed in Clonakilty (χ^2=8.58, df=2, P<0.05) and Oysterhaven (χ^2=7.31, df=2, P<0.05). Higher intensities of *Microphallus* spp. were recorded the middle site relative to the outer site in the case of Clonakilty (P<0.05) and relative to the inner site in Oysterhaven (P=0.05).

The intensity of *Microphallus* spp. significantly differed between sampling dates only in Clonakilty estuary, (χ^2=8.5, df=2, P<0.05). Higher intensities were recorded in August relative to June (P<0.05). Prevalence of *Microphallus* spp. in relation to sampling date only differed in Oysterhaven estuary with significantly lower prevalences recorded in August (χ^2=7.78, df=2, P<0.05). *Microphallus* spp. prevalence did not differ between sampling sites for Clonakilty and Oysterhaven estuaries.

Microphallus spp. prevalence and intensity were positively correlated (r=0.25, df=291, P<0.0001). Prevalence or intensity of *Microphallus* spp. did not differ

between sexes of *C. maenas.* However, *Microphallus* spp. intensity positively correlated with *C. maenas* weight (r=0.21, df=285, P<0.001) and carapace width (r=0.15, df =289, P<0.05).

Hediste diversicolor

Nematodes were found in 1%, 5% and 0.4% of *H. diversicolor* sampled from Clonakilty, Oysterhaven and Youghal, respectively (Fig. 4). 2% of *H. diversicolor* from Oysterhaven and Youghal contained copepods. The least common parasitic taxa in *H. diversicolor* were the cestode, *Ophryocotyle proteus* (Friis, 1870) and the halacarid mite with a prevalence of 0.15%. The cestode *O. proteus* and the halacarid mite were recorded in Clonakilty in two separate *H. diversicolor*.

Echinostomid metacercariae were significantly more prevalent in Clonakilty estuary than other estuaries(χ^2=84.00, df=2, P<0.001). 32.7% of *H. diversicolor* screened from Clonakilty, 10.8% from Oysterhaven and 3.3% from Youghal were infected by the echinostomid. The intensity of the echinostomid metacercariae was significantly higher in Clonakilty than in Youghal estuary (χ^2=7.76, df=2, P <0.05). Prevalence of the gymnophallid significantly differed between sites (χ^2=22.29, df=2, P<0.001). Highest prevalences of the gymnophallid were recorded in Clonakilty with 10.4% of *H. diversicolor* screened infected. Low prevalence was recorded in samples from Oysterhaven (3.3%) and Youghal (2%) estuaries (Fig. 4).

The prevalence of the echinostomid trematode significantly differed between sites sampled in Clonakilty (χ^2=35.51, df=2, P<0.0001), Oysterhaven (χ^2=11.01, df=2, P<0.01) and Youghal (χ^2=31.71, df=2, P<0.0001) estuaries (Fig. 7). The prevalence of the gymnophallid also differed between sites in Clonakilty (χ^2=23.77, df=2, P<0.001), Oysterhaven (χ^2=6.13, df=2, P=0.05) and Youghal (χ^2=15.24, df=2, P<0.001) estuaries (Fig. 8). Prevalence of the echinostomid trematode was lower in

the outer (more coastal) site in Clonakilty, Oysterhaven and Youghal estuaries, relative to the inner (brackish) site. In Clonakilty and Youghal lower prevalence of the gymnophallid was recorded in worms collected from outer sites relative to the inner sites. In Oysterhaven, the echinostomid had lower prevalence and the gymnophallid was absent, in the middle site of the estuary. The intensity of both trematode taxa did not significantly differ between sites within the estuaries.

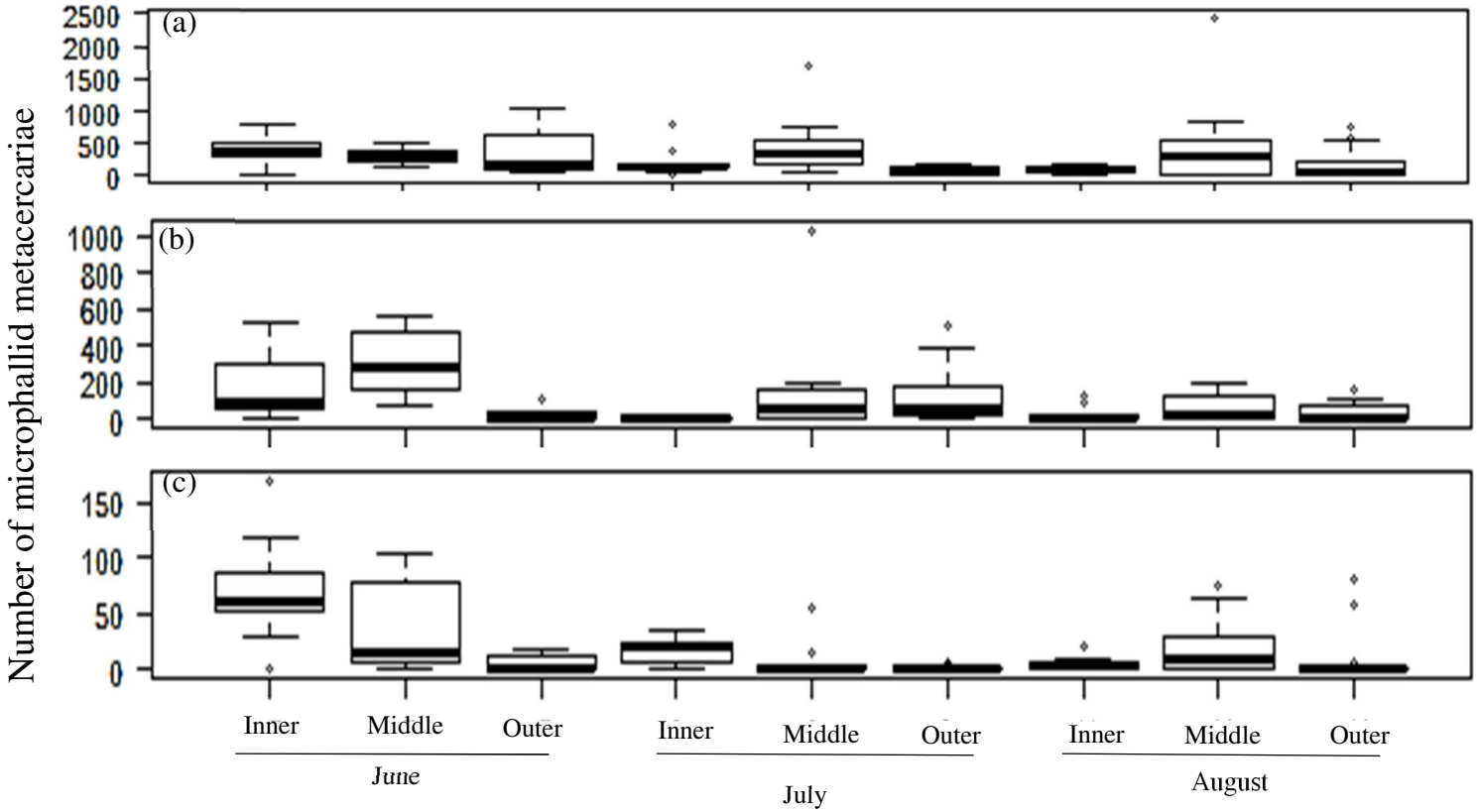

Fig. 6. Intensity of infection of *Carcinus maenas* by microphallid metacercariae in the sites within the estuaries (a) Clonakilty (b) Oysterhaven and (c) Youghal for the months of June, July and August 2015. Note the differing y-axes.

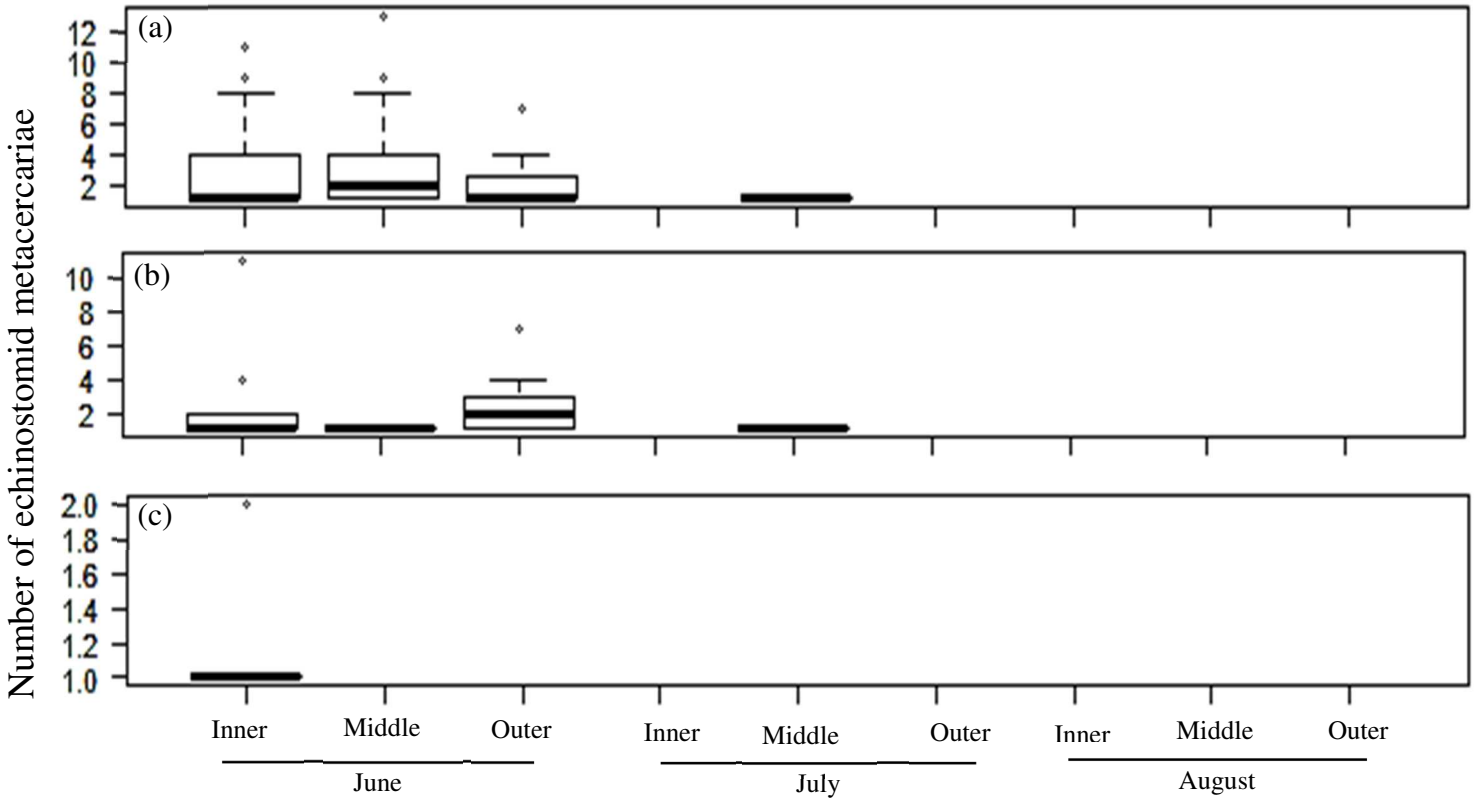

Fig. 7. Intensity of infection of *Hediste diversicolor* by echinostomid metacercariae in the sites within the estuaries (a) Clonakilty (b) Oysterhaven and (c) Youghal for the months of June, July and August 2015. Note the differing y-axes.

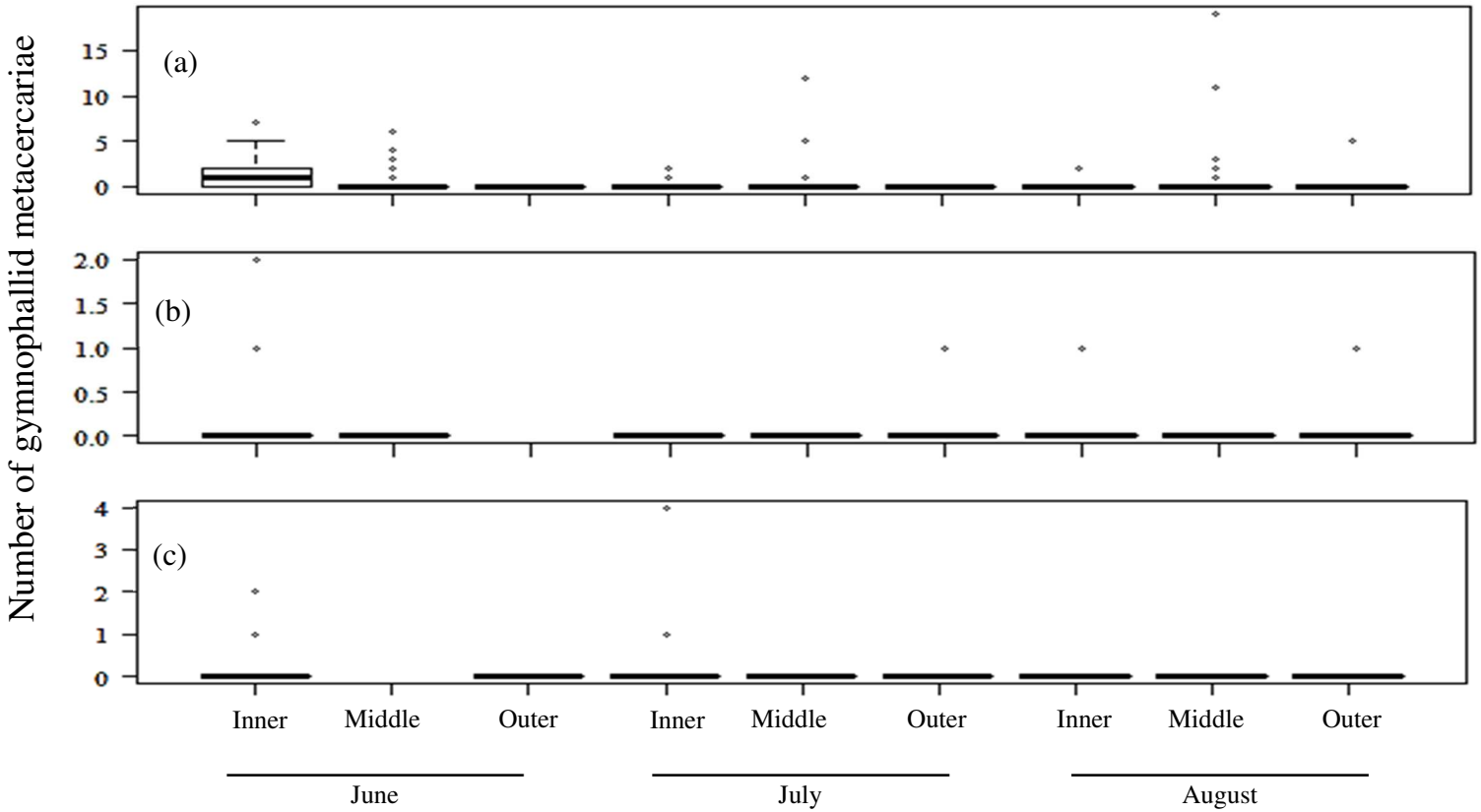

Fig. 8. Intensity of infection of *Hediste diversicolor* by gymnophallid metacercariae in the siteswithin the estuaries (a) Clonakilty (b) Oysterhaven and (c) Youghal for the months of June, July and August 2015. Note the differing y-axes.

4.3.2 Host morphometrics

Carcinus maenas

Carcinus maenas weight (χ^2=20.92, df=2, P<0.0001) and carapace width (χ^2=16.86 df=2, P<0.001) significantly differed between the three estuaries. *Carcinus maenas* sampled from Youghal estuary were larger than those sampled from Clonakilty (P<0.001) and Oysterhaven (P<0.05) estuaries. *Carcinus maenas* morphometrics also significantly varied between sites within Oysterhaven and Youghal estuaries. Larger *C. maenas* were sampled from the middle site in Oysterhaven (χ^2=8.74, df=2, P<0.05) and Youghal (χ^2=7.73, df=2, P<0.05) estuaries.

The sex ratio of *C. maenas* populations sampled did not significantly differ between estuaries, sites, or sampling events. The colour morphs of *C. maenas* significantly varied between estuaries (χ^2=21.07, df=2, P<0.0001). The green colour morph outnumbered the red morph in all estuaries (Table 1). In order of decreasing abundance, the red colour morph was found in Youghal, Oysterhaven and Clonakilty estuaries (Table 1). The occurrence of the red morph of *C. maenas* significantly differed between sitesin Clonakilty (χ^2=9.50, df=2, P<0.001) and Youghal estuaries (χ^2=16.80, df=2, P<0.001). The red morph of *C. maenas* was more common in the outer sampling site of Clonakilty estuary. In the case of Youghal estuary, the red morph was absent from the outer site.

Table 1 *Carcinus maenas* morphometrics averaged over all sampling events are presented as $\bar{x}$ (±SD). Male *C. maenas* is represented by m and female crabs by f.

Parameters	Estuaries		
	Clonakilty	Oysterhaven	Youghal
Weight (g)	10.63 (±7.4)	10.57 (±4.53)	14.03 (± 8.25)
Carapace width (mm)	34.62 (±7.89)	36.63 (±6.78)	38.36 (±7.86)
Sex ratio (m:f)	1:0.83	1:1.14	1:0.38
Colour morph ratio (red: green)	1:32	1:14	1:8

Hediste diversicolor

The wet weights of *H. diversicolor* did not significantly differ between estuaries (F=2.426, df=2, P>0.05) but did between siteswithin estuaries (see Table 2). In Clonakilty estuary, significantly heavier *H. diversicolor* were recorded in the outer site (χ^2=37.5, df=2, P<0.0001) relative to the inner and middle sites. In Oysterhaven estuary significantly heavier *H. diversicolor* were sampled from the middle site relative to the inner and outer sites (F=14.89, df=2, P<0.0001). In Youghal estuary heavier *H. diversicolor* were recorded in the outer site relative to the inner and outer sites (χ^2=11.81, df=2, P<0.05).

Table 2 *Hediste diversicolor* morphometrics averaged over all sampling events are presented as $\bar{x}$ (±SD). *Hediste diversicolor* with undifferentiated gonads are represented by u, males by m and female with f.

Parameters	Estuaries		
	Clonakilty	Oysterhaven	Youghal
Weight (g)	0.55 (±0.26)	0.53 (±0.33)	0.67 (±0.49)
Sex ratio (u:m:f)	1:0.18:0.25	1:0.84:0.54	1:0.36:0.26

4.3.3 Physico-chemical parameters

According to the nutrient parameters measured, Clonakilty estuary was the most nutrient rich estuary (Table 3). Results agreed with the WFD designation. Total phosphorous differed between estuaries (F=7.37, df=2, P<0.01) with significantly higher values recorded in Clonakilty (Tukey HSD, P<0.05). Ortho-phosphate significantly differed between estuaries (F=37.70, df=2, P<0.001) with higher values again recorded in Clonakilty (Tukey HSD, P<0.001). Levels of ortho-phosphate and

total phosphorous were highest in June and July at Clonakilty and exceeded threshold values for WFD classifications for unpolluted waters (O'Boyle *et al.,* 2010). Temperature also significantly differed between estuaries ($F=6.02$, $df=2$, $P<0.01$) with higher temperatures recorded at Clonakilty (Tukey HSD, $P<0.01$)

Table 3 Physico-chemical water parameters sampled from three sites within each estuary sampled for June-August 2015. Parameters are presented as $\bar{x}$ (±SD) and n=3 per site within estuaries sampled.

	Clonakilty			Oysterhaven			Youghal			Grand
Values	Inner	Middle	Outer	Inner	Middle	Outer	Inner	Middle	Outer	Total
Ammonia (mgL^{-1})	0.23 (±0.02)	0.04 (±0.01)	0.05 (±0.02)	0.04 (±0.01)	0.05 (±0.03)	0.03 (±0.01)	0.05 (±0.01)	0.06 (±0.02)	0.04 (±0.01)	0.07 (±0.06)
Ortho-P (mgL^{-1})	0.11 (±0.05)	0.03 (±0.02)	0.01 (±0.01)	<0.01 (±<0.01)	<0.01 (±<0.01)	<0.01 (±<0.01)	0.01 (±<0.01)	0.01 (±<0.01)	0.01 (±<0.01)	0.02 (±0.04)
Total Phosphorus (mgL^{-1})	0.32 (±0.22)	0.13 (±0.08)	0.06 (±0.02)	0.04 (±0.02)	0.05 (±0.01)	0.04 (±0.02)	0.13 (±0.09)	0.03 (±0.01)	0.04 (±0.01)	0.09 (±0.11)
Total Nitrogen (mgL^{-1})	2.19 (±0.77)	1.17 (±0.18)	0.76 (±0.21)	0.85 (±0.49)	0.90 (±0.36)	0.75 (±0.52)	1.56 (±0.52)	0.71 (±0.20)	0.62 (±0.05)	1.06 (±0.61)
Total Oxidised Nitrogen (mgL^{-1})	0.93 (±0.34)	0.12 (±0.09)	0.05 (±0.02)	0.27 (±0.31)	0.14 (±0.11)	0.06 (±0.05)	0.78 (±0.33)	0.24 (±0.15)	0.15 (±0.10)	0.30 (±0.35)
Salinity (%)	25.47 (±7.80)	28.07 (±5.50)	31.67 (±2.36)	28.90 (±2.38)	30.87 (±2.97)	31.13 (±2.32)	21.63 (±4.75)	28.77 (±3.39)	31.93 (±2.69)	28.71 (±4.75)
Turbidity (NTU)	8.67 (±3.21)	5.00 (±<0.01)	6.67 (±2.89)	10.33 (±9.24)	5.00 (±<0.01)	5.00 (±<0.01)	56.67 (±80.98)	6.67 (±2.89)	6.67 (±2.89)	12.30 (±27.79)
Temperature (°C)	21.90 (±2.77)	22.70 (±2.76)	18.07 (±1.62)	15.63 (±2.20)	16.27 (±0.23)	16.23 (±0.81)	19.20 (±4.35)	17.67 (±4.01)	19.13 (±6.03)	18.53 (±3.60)

4.3.4 Potential avian host surveys

There was no significant difference in avian richness between estuaries with 25 bird species recorded in Oysterhaven and 24 in both Clonakilty and Youghal (see Table 4). High abundances of birds were found in Clonakilty with 1, 249 individuals recorded, 561 individuals in Youghal and 264 in Oysterhaven. The abundance and distribution of species did vary between months with peaks seen in August. Simpson's Diversity index found Oysterhaven to have the most diverse bird community on average with an index of 1.69 (SD 1.45), followed by Youghal with 9.16 (SD 0.72) and Clonakilty with 6.79 (SD 2.04). Of the potential final hosts for helminth taxa recorded, gulls *Larus* spp. (Linnaeus, 1758), the ruddy turnstone *Arenaria interpres* (Linnaeus, 1758) and the Eurasian oystercatcher *Haematopus ostralegus,* only the abundance *H. ostralegus* significantly differed between estuaries with larger numbers recorded in Clonakilty ($F=19.42$, $df=2$, $P<0.05$).

Table 4 Avian taxa recorded in the three estuaries for the months June-August 2015 summed for all sampling events

Bird Taxa	Estuary			Taxa Total
	Clonakilty	Oysterhaven	Youghal	
Actitis hypoleucos, (Linnaeus, 1758) Common sandpiper	1	5	6	12
Anas spp. (Linnaeus, 1758) Dabbling ducks	39	23	8	70
Ardea cinerea (Linnaeus, 1758) Grey Heron	3	4	11	18
Arenaria interpres Ruddy turnstone	1	0	0	1
Bubulcus ibis (Linnaeus, 1758) Cattle egret	3	7	0	10
Calidris spp. (Merrem, 1804) Sand pipers	16	3	0	19
Carduelis carduelis (Linnaeus, 1758) European goldfinch	0	2	0	2
Charadrius hiaticula (Linnaeus, 1758) Common ringed plover	0	0	3	3
Columba palumbus (Linnaeus, 1758) Common Woodpigeon	0	13	0	13
Corvus spp. (Linnaeus, 1758) Crow species	58	40	30	128
Cygnus olor (Gmelin, 1789) Mute swan	48	0	0	48
Egretta garzetta (Linnaeus, 1766) Little egret	12	3	16	31
Haematopus ostralegus Oystercatcher	101	3	67	171
Hirundo rustica (Linnaeus, 1758) Barn swallow	0	4	5	9
Larus spp. Gulls	532	84	210	826
Limosa spp. Brisson, 1760 Godwit	247	27	48	322
Numenius spp. Moehring, 1758 Curlews	145	29	74	248
Phalacrocorax carbo (Linnaeus, 1758) Great cormorant	2	2	14	18
Sterna spp. (Linnaeus, 1758) Terns	0	0	26	26
Tadorna tadorna (Linnaeus, 1758) Common shelduck	11	0	8	19
Tringa spp. (Linnaeus, 1758) Shanks	30	15	35	80
Total number of individuals	1249	264	561	2074

Table 5 Correlation between trematode prevalence and final host abundance for all sites and sampling events in Summer 2015 for the three estuaries combined. Asterisk denote level of significance * for P<0.05, ** P<0.001 and *** P<0.0001

Trematode species	Final avian host	Correlation coefficient
Echinostomatid	*Anas* sp.	-0.13**
	Arenaria interpres	0.16***
	Calidris sp.	0.22***
	Haematopus ostralegus	0.06
	Larus sp.	-0.21
	Limosa sp.	0.36
	Numenius sp.	0.01
	Sterna sp.	-0.43**
	Tringa sp	0.07
Gymnophallid	*Anas* sp.	0.31***
	Haematopus ostralegus	0.24***
	Larus sp.	0.20***
	Sterna sp.	-0.27***
Microphallus spp.	*Anas* sp.	0.34***
	Haematopus ostralegus	0.03*
	Larus sp.	0.21***
	Tringa sp.	0.13*

Table 6 Correlation between trematode taxa and physico-chemical properties of estuarine water samples from all sampling points and events in Summer 2015 for all estuaries combined. NA represents not applicable as microphallid metacercariae were recorded in *Carcinus maenas* and echinostomid or gymnophallid metacercariae were recorded in *Hediste diversicolor.* Asterisk denote level of significance * for P<0.05, ** P<0.001 and *** P<0.0001.

	Echinostomatid	Gymnophallid	Microphallid	NH_3	PO_4^{3-}	TON	TP	TN	Salinity	Turbidity	Temperature
Echinostomatid	1										
Gymnophallid	0.13**	1									
Microphallid	NA	NA	1								
NH_3	0.02***	-0.15***	-0.15	1							
PO_4^{3-}	0.22***	-0.07	-0.15	0.52	1						
TON	0.11***	-0.24***	0.02	0.59	0.31	1					
TP	021***	-0.11***	0.48***	0.21	0.57	0.35	1				
TN	0.17***	-0.20***	0.33***	0.35	0.44	0.62	0.85	1			
Salinity	-0.03	0.02	-0.20 ***	-0.08	-0.03	-0.64	-0.21	-0.35	1		
Turbidity	-0.17***	-0.18***	0.02	0.42	0.40	0.56	0.31	0.28	-0.41	1	
Temperature	0.18***	0.07	0.11	0.25	0.62	0.30	0.56	0.52	-0.26	0.51	1

4.4. DISCUSSION

The study supports the hypothesis that eutrophication encourages macroparasite infection. The mechanisms by which eutrophication facilitates trematode infection are proposed to act at the juvenile stages (Johnson *et al.,* 2008). Excess nutrients are speculated to encourage intermediate host density and/or output of infective stages. No trematode infections preceding metacercariae, i.e. cercarial, were recorded during this study. However, the prevalence and intensity of metacercariae recorded in the enriched site indicates that trematode infections could be facilitated.

Higher abundances of avian hosts, and parasitic taxa were recorded at the eutrophic site, Clonakilty. In this study, trematode prevalence in both hosts was positively correlated with total Nitrogen and total Phosphorous concentrations. Nitrogen in salt marshes has been linked to increased trematode prevalence (Altman *et al.,* 2014). The differences in trematode intensity between estuaries could not be attributed to differences in host morphometrics. *Hediste diversicolor* did not significantly differ in size or between estuaries. Moreover, significantly lower intensity and prevalence of *Microphallus* spp. occurred in Youghal in which significantly larger *C. maenas* were screened. Stronger correlations were found with the proposed hosts for taxa found. Final hosts' distribution and movement are known to play a determining role in the distribution and prevalence of trematodes (Hechinger & Lafferty, 2005, Feis *et al.,* 2015).

Of the two invertebrate host species screened, *C. maenas* was the most commonly infected. Parasite communities were not taxa rich and were dominated by trematode metacercariae. The higher prevalence and intensity of *Microphallus* spp. is indicative of the trematode's ecological relevance and importance. The method of hand collection may produce high estimates of parasitic infection. Heavily infected *C. maenas* can be lethargic due to physiological stress and thus slower and, more easily

sampled by hand (Crothers, 1968, Werner, 2001). As a result, estimates of intensity of trematode infection in *C. maenas* sampled may be slightly higher than in the total population.

In all estuaries trematodes were the predominant helminth with high intensities of *Microphallus* spp. metacercariae in *C. maenas.* This finding agrees with the work of Zetlmeisl *et al.,* (2011) and Blakeslee *et al.,* (2015). Richness of macroparasite taxa recorded in *C. maenas* was lower than previous work in the United Kingdom and New Zealand but was consistent with histological work on the hermit crab, *Pagurus bernhardus* (Linnaeus, 1758) in Ireland (Stentiford & Feist 2005, Lynch *et al.,* 2015). Notably lacking from *C. maenas* sampled in this study was the rhizocephalan *Sacculina carcini,* Thompson, 1836*,* the turbellarian *Fecampia erythrocephala,* Giard, 1886, and the acanthocephalan *Profilicollis botulus* (Van Cleave, 1916). The absence of these taxa is likely due to the habitat and sizes of *C. maenas* sampled. *Sacculina carcini* is commonly found in subtidal habitats (Waser *et al.,* 2016). This distribution could be attributed to parasitic manipulation, with castrated or "feminised" *C. maenas* moving into the subtidal, like gravid females would. The subtidal habitat may facilitate the survival of *S. carcini* infective stages increasing the likelihood of them reaching and infecting a decapod (Rasmussen 1959, Werner, 2001, Waser *et al.,* 2016). Alternatively, the parasitic taxa could have eluded detection due to their small size or an early stage of infection. According to Kuris *et al.,* (2002), *F. erythrocephala* is commonly found in small *C. maenas* with carapaces less than 11mm in width.

Macroparasite communities of *C. maenas* warrant further study, as they play a role in the ecology and physiology of *C. maenas* (Zetlmeisl *et al.,* 2011). Microphallids, in particular *Microphallus similis,* affect the body mass index, energy storage, and immune activation of *C. maenas* in its invasive range (Blakeslee *et al.,* 2015). The escape of *C. maenas* from two thirds of its native parasites is considered key to the crab's invasive success (Torchin *et al.,* 2001). In the invasive range, *C. maenas* is not

only parasitised by fewer taxa but also to a lesser extent than individuals within its native range (Torchin *et al.,* 2001, Blakeslee *et al.,* 2015). The higher intensity of *Microphallus* spp. may have implications for the health of *C. maenas.* Heavy microphallid infections in the crab *Neohelice granulata* (Dana, 1851) resulted in structural damage to the hepatopancreas, necrosis of digestive tubules and impaired physiological storage (Robaldo *et al.,* 1999). Heavy infection by microphallid metacercariae in *C. maenas* in the field impaired the crab's self-righting ability (Blakeslee *et al.,* 2015) and survival under laboratory conditions (Stunkard 1957).

Hediste diversicolor was found to harbour greater parasite richness than *C. maenas* in the three estuaries surveyed. *Hediste diversicolor* was used as a second intermediate host by two trematode taxa (Margolis, 1971; 1973). Polychaetes in New Zealand were primarily infected by a single trematode species in the form of metacercariae (Peoples *et al.,* 2012). High intensities of metacercariae, 18 per worm, impaired the structural integrity of New Zealand polychaetes and led to fragmentation of the worm's body (Peoples *et al.,* 2012). If metacercariae have similar effects on *H. diversicolor,* it could affect their economic value as a bait fishery (Torchin *et al.,* 2001, Peoples *et al.,* 2012).

The effect of transient algal mats in Clonakilty on host-trematode dynamics in estuaries, is likely complex. Algal mats may increase local density of intermediate invertebrate hosts (Hull, 1987, Lewis *et al.,* 2014). Dense mats of vegetation can be important feeding areas for birds that either feed directly on the vegetation or on invertebrates found within the mats (Goss-Custard *et al.,* 2006). Algal mats may favour trematode taxa by increasing the abundance of their common primary intermediate host the Laver spire shell *Peringia ulvae* (Pennant, 1777), formerly *Hydrobia ulvae*. *Peringia ulvae* can colonise and consume transient algal mats that are present in Clonakilty estuary (Soulsby *et al.,* 1982, Hull, 1987). *Peringia ulvae* is also a host for a large range of trematode species including *Microphallus* spp. and echinostomatids (Stunkard, 1957, Deblock 1980). Proximity of trematode hosts is vital

for the taxa's transmission and the increased density of primary intermediate trematode hosts is likely to facilitate high prevalence of *Microphallus* spp. recorded (Fredensborg & Poulin, 2006).

The infective stage preceding the metacercaria, the cercaria, is short lived, free living and mobile. It is usually released into the water column to encounter and infect a suitable host and continue its life cycle. Under laboratory conditions, macroalgae can impair the dispersal and successful infection of cercariae (Welsh *et al.,* 2014). This could decrease the intensites of metacercarial infections as the local abundance of cercariae determines the encounter rates of the trematode with their crustacean intermediate hosts (Thieltges *et al.,* 2009). However, the high intensities of *Microphallus* spp. recorded in Clonakilty suggest that any physical disturbance of infective stages by algae, is overcome by increased densities of gastropod hosts.

Eutrophication from organic sources has been recorded to favour gulls (Raven & Coulson 2001). The effect of algal mats on avian hosts varies in magnitude and in relation to the avian species. Soulsby *et al.,* (1982) suggested that the effect was small and several prey items decreased due to covering or algal decomposition could be compensated by increased densities of the gastropod *P. ulvae*. In Clonakilty estuary, the effect of algal mats on birds is species-specific with black backed gulls, *Larus* marinus, foraging in algal cleared areas (Lewis *et al.,* 2014). Algal mats may also affect the foraging strategy of bird species within the estuary. Californian shorebirds switch from foraging pecks to probes in response to increased coverage by algal mats in eutrophic estuaries (Green *et al.,* 2015). If bird communities in Irish estuaries adopt the same foraging behaviour, this could explain the higher prevalence and richness of *H. diversicolor* in Clonakilty than the less eutrophic estuaries sampled. Algal mats may affect the density of *Hediste diversicolor.* However, the effect may vary as previous work has found that the abundance of *H. diversicolor* was greater (Hull,

1987; Norkko & Bonsdorff, 1996b) and less abundant (Lopes *et al.,* 2000) under algal mats relative to uncovered areas of mudflat.

The occurrence of trematodes in all estuaries surveyed was high. However, greater richness, prevalence and intensity of parasitic taxa was found in the eutrophic estuary of Clonakility. In the case of trematodes, this could be due to an increased output of infective stages by molluscan and/or avian hosts. There was a positive correlation between enrichment parameters, phosphorous and nitrogen, and trematode prevalence. This was a correlative field study, so interpretation and extrapolation of results was conservative. Observed patterns of trematode distributions could be confounded by additional, unmeasured variables e.g. heavy metal concentrations or differing invertebrate host abundances (Mischler *et al.,* 2016). Human infection by trematode taxa in this study is possible but likely harmless. Cercariae from trematodes that utilise birds as final hosts, may encounter and infect the skin of a swimmer. Dermatitis or "swimmers' itch" results from the penetration of cercariae into skin and the subsequent irritation, arrested maturation or death of cercariae (Fraser *et al.,* 2009, Horak *et al.,* 2015). However, the dermatitis is generally temporary, due to the short lifespan of the cercariae (<24hrs) and the immune-physiology of human skin (Fraser *et al.,* 2009, Horak *et al.,* 2015, Thieltges *et al.,* 2006b).

The high prevalence of trematodes in all estuaries sampled is indicative of their ecological relevance. If the hypothesis is correct and the recorded higher trematode intensity is causally related to increased nutrient parameters, there may be implications for the survival and ecological interactions of invertebrates screened. Future work should consider manipulative laboratory trials to test the mechanisms of how eutrophication may facilitate parasitism in estuaries. Laboratory trials by Johnson *et al.,* 2007 with the freshwater snail species composition *Planorbella* spp., *Ribeiroia ondatrae* and . Additionally, the effect of trematode metacercariae on the physiology and behaviour of invertebrate hosts screened warrants investigation. This study reports

the detection of trematode metacercariae in *Hediste diversicolor* and raises the question as to what impact this parasite may be having on populations of the worm in estuaries on the southwest coast of Ireland.

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CHAPTER 5: Parasites and pathologies of the blue mussel (*Mytilus* spp.): the role of host stress in susceptibility

ABSTRACT

The following study was undertaken to investigate the relationship between parasite communities of the blue mussel, *Mytilus* spp. and water quality in areas where the bivalve was found. A year-long survey assessed parasite communities of two subtidal *Mytilus* spp. populations inside and outside a marine reserve, Lough Hyne Co. Cork. It was hypothesised that *Mytilus* spp. outside the reserve would experience higher prevalence and intensity of parasite infection due to increased exposure to anthropogenic stressors. From the 731 *Mytilus* spp. screened nine parasite taxa were recorded: four macroparasites and five microparasites, including apicomplexans gregarines, ciliates, prokaryotic inclusions, *Rickettsia*-like organism /*Chlamydia*-like organisms, trematodes, nematodes, copepods and a cestode. Copepods and gregarines were found to be the most common macroparasite and microparasite, respectively. Data were analysed in relation to sex, gonadal development stage, condition index and the presence of parasites. Month, sex of *Mytilus* spp. and site sampled were not important determinants for the observed parasite prevalence or intensity. Prevalence of parasites did not correlate with pathologies recorded. Lipofuscin accumulation in tissues was the most common pathology recorded in *Mytilus* spp. and varied in relation to month of sampling. The low prevalence and intensity of trematode taxa were recorded in both *Mytilus* spp. populations in the field study was investigated with a series of laboratory trials assessing the susceptibility of *Mytilus* spp. to the trematode *Echinostephilla patellae.* To investigate the role of host stress in susceptibility, *Mytilus* spp. were sampled from habitats of differing levels of exposure to air, a bloom of *Karenia mikimotoi*, and to simulated nutrient enrichment. The findings of the laboratory trials were inconclusive, due to the high level of natural trematode infections of the 240 specimens included in the trials. There was a general, but insignificant trend, of increased *E. patellae* prevalence in *Mytilus* spp. exposed to

cercariae. It was not possible to distinguish between pre-existing natural and laboratory infections of *E. patellae* due to the inability to age metacercariae based on morphology and current knowledge. Overall, the study suggests *Mytilus* spp. are not negatively affected by recorded parasite taxa and intensity of infection under current environmental conditions and water quality classifications.

5.1. INTRODUCTION

Mussels from the genus *Mytilus* are widely used in biomonitoring programs such as "Mussel watch" (Goldberg, 1975, Borja *et al.,* 2008, Kim *et al.,* 2008). Taxa used in biomonitoring programs are generally well studied, abundant, sedentary, have high tolerances to physiological stresses and wide global distributions. *Mytilus* are distributed from the subarctic to temperate regions, are sessile, resistant to pollution, euryhaline and can accumulate metals at 10^3 to 10^5 higher concentrations than the surrounding environment (Gosling, 2003). *Mytilus* spp. are filter feeders and can also concentrate pollutants, allow them to act as an early warning system of ecosystem decline or anthropogenic impacts (Gosling, 2003).

Mytilus spp. are ecosystem engineers in the rocky intertidal providing habitat through their shells or the dense aggregations that they form. Patches of *Mytilus* spp. can provide refuge and habitat for a wide variety of associated organisms in the rocky intertidal (e.g. Seed, 1996, O'Connor & Crowe 2007; 2008). *Mytilus* spp. are also hosts to a variety of different parasite taxa, including shell boring polychaetes, intestinal copepods, and trematodes (Lauckner, 1983). Work on Irish rocky shores and UK estuaries found trematodes to be common parasites of *Mytilus* spp. (Bignell *et al.,* 2008, Prinz *et al.,* 2010a, Lynch *et al.,* 2014). In the North Atlantic, blue mussels are commonly found to be second intermediate hosts of the trematode genera *Gymnophallus*, *Himasthla* and *Renicola* (Galaktionov*et al.,* 2015). Trematodes that utilise mussels as primary hosts are usually from the Bucephalid family; *Prosoryhnchus squamatus* (Odhner, 1905) and are found predominantly in Northwestern Europe, Britain and Atlantic Canada (Lauckner 1983, Coustau *et al.,* 1991).

Histopathology can assess the pathological, reproductive and metabolic condition of individual *Mytilus* spp. simultaneously. Tissue pathologies in *Mytilus* spp. have been

used in a wide range of studies to evaluate individual populations and their surrounding environment's health (Svärdh *et al.,* 2002, Aarab *et al.,* 2008, Bignell *et al.,* 2008). In response to increasing concentration of contaminants and heavy metals, increased levels of the age pigment (lipofuscin) and inflammatory lesions have been recorded in *Mytilus* spp. (Aarab *et al.,* 2008, Bignell *et al.,* 2011, Brenner *et al.,* 2014). Accumulation of lipofuscin granules, a type of insoluble cellular biomarker, can reflect the sublethal effects of pollutants and cell damage (Riveros *et al.,* 2002). Metal detoxification can be aided by lipofuscin granules (Riveros *et al.,* 2002; Dimitriadis *et al.,* 2004). Histopathological surveys of *Mytilus* spp. in Ireland, the UK and USA have found the bivalve to commonly harbour trematode taxa (Bignell *et al.,* 2008, Bignell *et al.,* 2011, Lynch *et al.,* 2014, Powell *et al.,* 2015a; 2015b). Studies that consider the whole specimen with dissection or compression techniques have also found this to be the case in the Wadden, North Atlantic, Pacific and Arctic Seas (Thieltges *et al.,* 2006a, Prinz *et al.,* 2010a, Wilson *et al.,* 2013).

Parasite communities of *Mytilus* species have previously been used as indicators for environmental quality (Kim *et al.,* 2008; Sures, 2008, Brenner *et al.,* 2014). Parasite diversity and abundance in mussels have been linked with poor water quality in previous studies in Ireland, Sweden and the UK (Svärdh, 1999; Svärdh and Johannesson, 2002; Bignell *et al.,* 2008, Lynch *et al.,* 2014). Predicted environmental changes have been shown to affect the immuo-competence of *Mytilus* spp. which may affect their susceptibility to parasites (Brenner *et al.,* 2014, Mackenzie *et al.,* 2014a; 2014b). Pollution can decrease the abudnance of parasites depending on its effect on the host and the parasite taxa in question (Sures, 2008, Sures *et al.,* 2017). Also it should be noted that harbouring parasites is not necessarily equated to poor health of individuals or ecosystems (Hudson *et al.,* 2006).

The effect of eutrophication on the susceptibility of *Mytilus* spp. was also of interest. Eutrophication is expected to broadly facilitate trematode infection by increasing the

abundance of gastropod hosts and the production of infective stages (Johnson *et al.,* 2010, Sures *et al.,* 2017). Finally, three intertidal populations of *Mytilus* spp. experiencing high, moderate and low exposure to a bloom were sampled from shores increasingly distant) from a bloom of *Karenia mikimotoi* (Miyake & Kominami ex Oda) Gert Hansen & Ø.Moestrup, 2000.

The first aim of this study were to examine if populations of *Mytilus* spp. could be characterised by their histopathology and parasite communities within and outside a marine reserve. Subtidal mussels were sampled as they may readily reflect water quality being constantly immersed and potentially continuously filtering. The survey was carried out over a year to account for seasonal variation in *Mytilus* spp. physiology and reproduction (Bignell *et al.,* 2008).

Laboratory trials were then used to investigate the role of host stress on *Mytilus* spp. and its susceptibility to the trematode *Echinostephilla patellae. Echinostephilla patellae* was chosen as it is an abundant trematode on the southwest coast of Ireland and has been experimentally established as a parasite of *Mytilus* spp. (Prinz *et al.,* 2010a; 2010b). The susceptibility of intertidal and subtidal populations of *Mytilus* spp. to *E. patellae* was of interest. The intertidal zone of rocky shores is physiologically stressful, as biota experience large changes in temperature, moisture and physical disturbance by waves. *Mytilus edulis* from or translocated to the intertidal zone have been recorded to have wider physiological tolerances than subtidal individuals (Altieri, 2006, Tagliarolo *et al.,* 2012). It was hypothesised that subtidal *Mytilus* spp. would show a higher occurrence of infection.

5.2. MATERIALS AND METHODS

5.2.1 Parasite Field Survey study sites

The study sites were three mooring lines on the southwest coast of Ireland (see Fig. 1). In Lough Hyne marine reserve, *Mytilus* spp. were sampled in the North Basin (51°30'06'0" N 9°18'02.5" W). Tranabo bay (51°29'52.7"N 9°17'07"W) is a small southern facing shore found to the east of the reserve. Cunnamore pier (51°30'21.1"N 9°25'22.3"W) is located within the small and sheltered Rincolisky Bay enclosed behind Heir Island and Sherkin Island. To have a replicate subtidal population of *Mytilus* spp. to sample, a buoy was deployed ca. 5km to the west of Lough Hyne. However, the buoy failed to recruit *Mytilus* spp.

5.2.1.2 Parasite Field Survey Sampling of *Mytilus* spp.

Mytilus edulis (Linnaeus, 1758) and *Mytilus galloprovincialis* (Lamarck, 1819) hybridise on shores along the southwest coast of Ireland (Gosling *et al.,* 1981; 2008). Distinguishing between the different *Mytilus* species, based on morphology, is problematic as no factor to date can consistently differentiate between the two species. Molecular screening is needed to differentiate between the two species and their hybrids based on the Me15/16 gene (Coughlan & Gosling, 2007). The gene encoding a polyphendic adhesive protein which is a key component in the attachment of mussels to substrates. These markers allow for the reliable estimation of the numbers of parental and hybrid mussel classes within hybrid zones (Inoue *et al.,* 1995). Thus, in this study, the mussels *M. edulis* and *M. galloprovincialis* are grouped into the genus *Mytilus* spp. Coustau *et al.,* (1990) suggested *M. edulis* species were more susceptible to the trematode *Prosorhynchus squamatus,* Odhner, 1905, relative to hybrids of *M. edulis* and *M. galloprovincialis*. However, more recent work has found no significant difference between the species and their hybrid in terms of susceptibility to parasite

infection (Bignell *et al.,* 2008). In Ireland, higher diversity and prevalence of parasites were recorded in *M. edulis* relative to *M. galloprovincialis* but no evidence of increased susceptibility of the species' hybrid was found (Lynch *et al.,* 2014).

Subtidal *Mytilus* spp. were sampled on a monthly basis from April 2013 until April 2014 in Lough Hyne and Tranabo bay. A once off sample was taken from a mooring line in Cunnamore pier, as after sampling the line was cleared of subtidal *Mytilus* spp. For all sampling events of subtidal *Mytilus* spp. specimens were sampled haphazardly by hand from mooring lines in the first 1-2 m below the waters' surface. *Mytilus* spp. <40 mm in shell length were not collected as smaller sizes are generally not parasitised (Bignell *et al.,* 2008; 2011). *Mytilus* spp. were then transported to the laboratory and processed for histology within 48 hours of collection.

5.2.1.3 Parasite Field Survey Methodology

The soft tissue of each mussel was dissected within 24 hours of collection from the fresh individuals. Prior to their dissection, the shell length, width and weight of individual *Mytilus* spp. were measured to the nearest 0.05 mm with vernier callipers. Adductor muscles were severed and soft tissue was removed from the shell. For each *Mytilus* spp. sampled, a section of tissue containing gill, gut, gonad and foot tissue, approximately 1 cm, in width was excised. The tissue section was then fixed in Davidson's solution at 4 °C for 48h as outlined by Shaw and Battle (1957). After 48 h in 70% ethanol, tissue was dehydrated through an ascending ethanol series in a Shadon Tissue Processor Citadell 1000. Tissue was embedded in paraffin wax, cut in 5-7 µm slices with a Leica RM2235 microtome. Tissue sections were placed on clean standard microscope slides and dried overnight in an oven. Slides were then stained using haematoxylin & eosin in a Leica ST5010 Autostainer XL. Slides were mounted in DPX (Distrene, Plasticiser, Xylene). After a week examined under x 200 or 400 magnifications with transmitted light to ensure the mounting medium was set.

Parasitological terminology and microscopes follows the specifications of Chapter 2 Section 2.2.3 page 43.

5.2.1.4 Parasite Field Survey analysis

Histopathological analysis was carried out according to the protocol outlined by Kim *et al.,* (2006). Parasites such as ciliates, prokaryotic inclusion bodies (PIBS), nematodes, gregarines, cestodes and trematode metacercariae were counted per tissue type. PIBS were counted when a dark staining intracellular inclusion 5-10 µm in size was seen within a tissue. Pathologies listed in Kim *et al.,* (2006) were qualitatively recorded per tissue type examined. Each cross section of a nematode or a copepod was counted as a single individual. The sex and gonadal stage of specimens were noted, based on scales modified from Seed (1969). Four stages of gonadal maturity were identified: resting, developing, ripe and spawning.

5.2.2 Laboratory trial studies

Three laboratory trials were carried out to investigate the role of host stress on *Mytilus* spp. and its susceptibility to *Echinostephilla patellae* cercariae, collected from its intermediate host *Patella vulgata* Linnaeus, 1758 (see Fig. 2 for experimental designs). Mortality was considered when the shell was gaping and the mantle was unresponsive to mechanical stimulation. Attempts were made to recover cercariae that failed to infect *Mytilus* spp. from tanks. 48 hours after exposure to cercariae whole mounts of *Mytilus* spp. were examined for parasite taxa. Prior to examination shell length, width and weight of individual *Mytilus* spp. were measured to the nearest 0.05 mm with Vernier callipers. Whole tissue mounts were compressed between ¼ inch thick glass slides and examined with microscopes detailed in Chapter 2, Section 2.2.3, page 43. The foot of *Mytilus* spp. was examined separately due to the differing density

of the tissue and recorded preference of *E. patellae* to infect the tissue (Prinz *et al.,* 2009). All parasites encountered were recorded in tissue specific counts.

Trial 1 (T1) investigated the role of differing levels of physiological stress experienced by *Mytilus* spp. in the intertidal and subtidal zone. It was hypothesised that the abiotic conditions of the intertidal zone may be physiologically demanding relative to the subtidal, thus intertidal *Mytilus* spp. would be more susceptible to trematode infection. Trial 2 (T2) investigated three intertidal populations of *Mytilus* spp. experiencing high, moderate and low exposure to a bloom of *Karenia mikimotoi*. Concentrations of *K. mikimotoi* reached 729,000 cells $-^{1}$ at the height of the bloom and were roughly 12,000 cells L^{-1} at the time of sampling. Previous blooms of *K. mikimotoi* in Ireland and Scotland resulted in mass mortality (>90%) of invertebrate populations through a combination of hypoxia and toxicity (Raine *et al.,* 2001, Davidson *et al.,* 2009). *Karenia mikimotoi* produces haemolytic toxins that supress *Mytilus* spp. immune function and influence bivalve growth and mortality (Matsuyama *et al.,* 1999, Hégaret *et al.,* 2011). Thus, *Mytilus* spp. populations that experienced greater exposure due to their higher proximity to the bloom *K. mikimotoi* were hypothesised to be more susceptible to *E. patellae* (Matsuyama *et al.,* 1999).

Trial 3 (T3) investigated the effect of acute host exposure to enriched nutrient conditions on host susceptibility. *Mytilus* spp. in higher nutrient concentrations were hypothesised to be more susceptible to *E. patellae,* due to conditions favouring cercariae survival and infectivity (Zander and Reimer, 2002, Johnson *et al.,* 2010, Johnson & Carpenter, 2008). *Mytilus* spp. were sampled from an intertidal population on the east coast previously sampled and found to be free of *E. patellae* infection by Prinz *et al.,* (2010b).

5.2.2.1 Laboratory trial collection sites

For all trials, *Patella vulgata* were sampled from the intertidal zone of Bullen's Bay, a sheltered rocky shore on the southwest of Ireland (51°38'37.0"N 8°33'19.0"W). For T2 *Mytilus* spp. were collected from the intertidal zone of three rocky shores, the moderately wave exposed Ringaskiddy (51°49'54.4"N, 8°18'04.0"W), the sheltered shore Cusheen (51°31'43"N, 9°31'34"W), and the North Shore in Lough Hyne (51°30'21"N, 9°18'12"W). The distance of the site from highest recorded concentration of *K. mikimotoi* cells was a proxy for decreasing exposure, in order of proximity Cusheen (ca. 15km), Lough Hyne (ca. 30km) and Ringaskiddy (ca. 100km). For T3 *Mytilus* spp. were collected from the moderately exposed rocky shore Blackrock (53°18'00"N 6°10'03"W) Co. Dublin on the east coast of Ireland.

5.2.2.2 Laboratory trial study collection of mussels

Mytilus spp. >4cm in length were sampled haphazardly by hand at low tide from all sites. In November 2015 intertidal and subtidal *Mytilus* spp. were collected in Lough Hyne from the intertidal zone and from mooring lines in the first 1-2m below the waters' surface as described previously in Section 5.2.1. In September 2015 intertidal *Mytilus* spp. were sampled from Cusheen, Lough Hyne and Ringaskiddy for T2. In October 2015 intertidal *Mytilus* spp. were sampled at low tide for T3. 100 intertidal *P. vulgata* were sampled haphazardly by hand at low tide in Bullen's Bay for on the first day of the trial for each run of laboratory trials.

5.2.2.2 Laboratory trial study experimental design

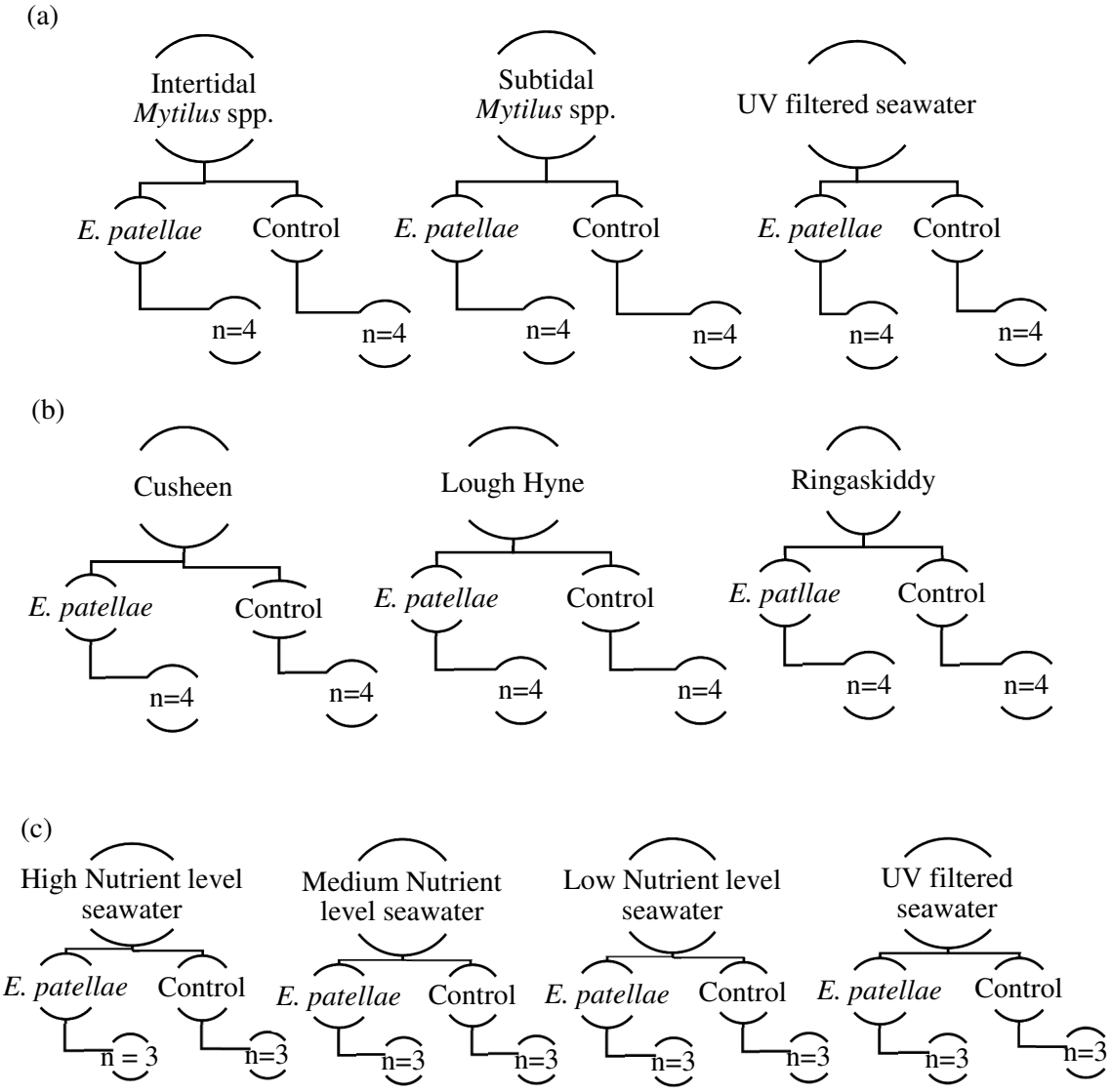

Fig. 2 Schematic diagram of experimental design of the *Mytilus* spp. challenge experiments (a) Trail 1: Host habitat and susceptibility to trematode infection (b) Trial 2: Red tide susceptibility trial (c) Trial 3: Acute exposure nutrient enrichment and host susceptibility trial. Three experimental runs of each trial were carried out.

5.2.2.3 Laboratory trial study experimental protocols

T 1 Host habitat and susceptibility to trematode infection trial

Prior to the commencement of the trial epibionts were removed from *Mytilus* spp. However, *Mytilus* spp. were not cleaned and over the duration of the trial the water in the tanks water was not changed. Intertidal and subtidal *Mytilus* spp. were placed in two plastic aerated tanks (30 X 20 X 20 cm) filled with UV-filtered seawater. Tanks were in were kept within a climate change chamber at 15 °C with 12:12 light: dark photoperiod. *Mytilus* spp. were randomly and individually placed in glass tanks (Fig. 3(c)) with 300 ml of UV-filtered seawater 24 hours prior to exposure to *E. patellae* cercariae collected from *P. vulgata* (Fig. 3(b)). *Mytilus* spp. in assigned tanks had acclimatised for 24 h prior to exposure to cercariae. Each of the three treatments was replicated 4 times per experimental run (Fig. 2a). Water from replicate tanks were filtered with a 20 µm sieve.

T 2: Red tide susceptibility trial

Prior to the commencement of the trial the epibionts were removed from *Mytilus* spp. However, *Mytilus* spp. were not cleaned and over the duration of the trial and the water in tanks was not changed. *Mytilus* spp. from the three sites sampled were placed in three separate plastic aerated tanks (30 X 20 X 20 cm) filled with UV-filtered seawater and maintained in the same environmental conditions the previous trial. Individual *Mytilus* spp. from Cusheen, Lough Hyne and Ringaskiddy were randomly and individually placed in glass tanks with 300 ml of UV-filtered seawater 24 h prior to exposure to *E. patellae* cercariae collected from *P. vulgata*. Each of the three treatments was replicated 4 times per experimental run. Water from replicate tanks were filtered with a 20 µm sieve.

T 3 Acute exposure nutrient enrichment and host susceptibility trial

Prior to the commencement of the trial the *Mytilus* spp. epibionts were removed from *Mytilus* spp. However, were not cleaned and over the duration of the trial the in the tanks water was not changed. *Mytilus* spp. sampled from Blackrock were randomly assigned to different treatments of nutrient concentrations of either 'Low' (7 µM phosphate, 140 µM nitrate and 2.6 µM nitrite), 'Medium' (15 µM phosphate, 278 µM nitrate and 5.14 µM nitrite) or 'High' (30 µM phosphate, 556 µM nitrate and 10.28 µM nitrite) (Fig. 2(c)). Concentrations were chosen based on the work of Malham *et al.,* (2009), varying amouny of Phosphate (PO_4^{3-}), Nitrate (NO_3^{1-}), and Nitrite (NO_2^-), were added to high, medium and low treatments see Table 2. In each plastic tank (30 X 20 X 20 cm) 25 *Mytilus* spp. were kept in a 7.5L of UV treated seawater with added nutrients or without in the case of control tanks. *Mytilus* spp. were maintained in plastic tanks at the same environmental conditions as T1 and T2 for a week and fed with 9.41ml of Reed Shellfish Diet 1800® (approx. 2 billion cells ml^{-1}) twice in that period. *Mytilus* spp. were then randomly and individually placed in glass tanks with 300ml of UV-filtered seawater 24 h prior to exposure to *E. patellae* cercariae collected from *P. vulgata*. Water from replicate tanks were filtered with a 20µm sieve.

Table 1. Nutrient levels proposed based on Malham *et al.,* (2009). All values are given as mgL-1

Chemical parameter	High	Medium	Low	Total per run
Sodium Phosphate monobasic dihydrate (NaH_2PO_4)	2.85	1.42	0.66	4.40
Sodium Nitrate ($NaNO_3$)	517.12	17.24	8.68	543.04
Sodium Nitrite ($NaNO_2$)	0.47	0.24	0.12	0.83

Echinostephilla patellae cercariae collection from infected *Patella vulgata*

Echinostephilla patellae cercariae were obtained from infected *Patella vulgata,* based on work by Prinz *et al.,* (2009; 2010b). Prior to storage in dry darkness for an hour at ca. -4°C, epibionts were removed from the limpets' shells to avoid physical disturbance or impairment of *E. patellae* cercariae (Prinz *et al.,* 2011). *Patella vulgata* were then immersed in 30ml of UV-filtered seawater in plastic petri dishes under bright light (Fig. 3 (a)). *Echinostephilla patellae* cercariae were shed from *P. vulgata* utilised as primary intermediate hosts. One *P. vulgata* utilised as a primary

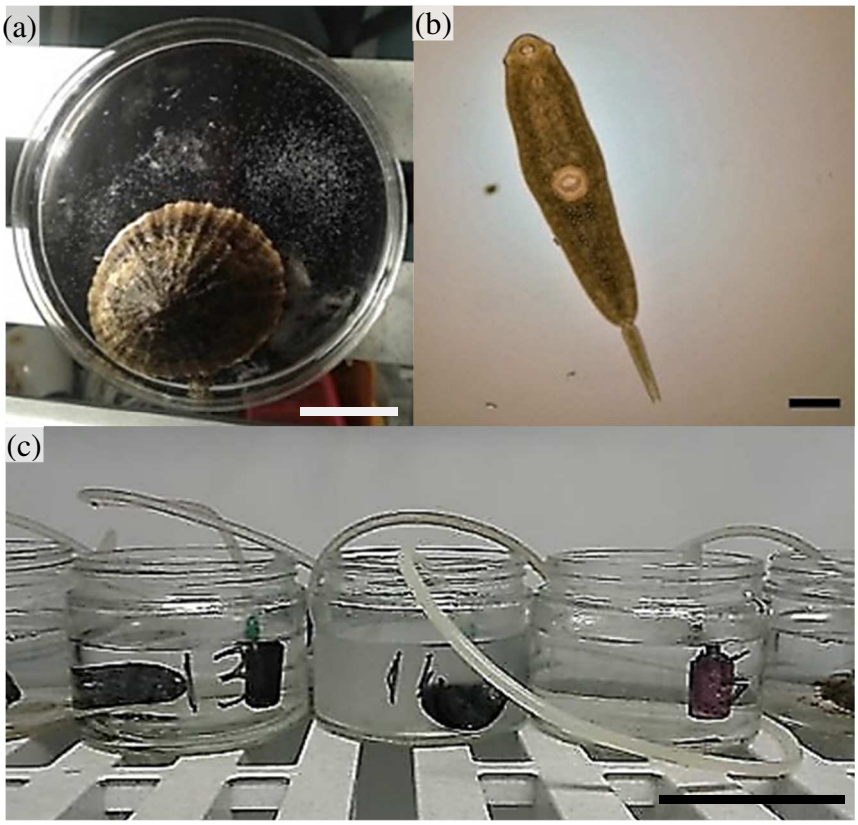

Fig. 3. Experimental set up of obtaining cercariae (a) Primary intermediate host *Patella vulgata* "sheds" *Echinostephilla patellae* cercariae in 30ml of UV filtered seaweater. (b) *Echinostephilla patellae* cercariae (c) *Mytilus* spp. in aerated glass tanks of 300ml UV-filtered seawater to which cercariae were pippetted. Scale bars indicates (a) 2.25cm and (b) 200µm (c) 9cm.

intermediate host by *E. patellae* was selected from the 100 screened to collect standard doses of cercariae from, either 10 or 20 individuals. *Echinostephilla patellae* cercariae were then pipetted into assigned glass tanks of 300ml UV filtered water (see Fig 1).

5.2.3 Statistical Analysis

Analysis was carried out in R studio version 1.0.136 (R Core Team 2017). Statistical significance was considered with $P<0.05$. The field study was 2 factor analysis with the location (fixed, orthogonal, 2 levels) and month sampled (fixed, orthogonal, 12 levels) considered factors. The laboratory study was a series of two factor analyses. In T1 the factors were habitat (fixed, orthogonal, 2 levels), and *E. patellae* exposure (fixed, orthogonal, 2 levels) (See Fig. 2 (a)). In T2 location (random, orthogonal, 3 levels) and *E. patellae* exposure (fixed, orthogonal, 2 levels) were the factors considered (See Fig. 2 (b)). In T3 the nutrient level (fixed, orthogonal, 4 levels) and *E. patellae* exposure (fixed, orthogonal, 2 levels) were the factors considered (See Fig. 2 (c)).

5.2.3.1 Parasite data analysis

Differences in parasite prevalences were tested with a Pearson's chi square with Yates continuity correction. If expected values were too low (<5) for a Pearson's chi square, a Fisher's Exact test was used. A Kruskal-Wallis test was carried out to examine differences in parasite intensities between sites, seasons, and sex. Pairwise Wilcoxon Rank Sum Tests was performed if significant differences were found. Parasitology terms follows Bush *et al.,* (1997).

5.2.3.2 Host data analysis

Host size characteristics between populations were tested with an Analysis of variance (one-way ANOVA) in combination with the posthoc test Tukey's honestly significant difference (HSD) test. Prior to the ANOVA being carried out, assumptions of homogeneity of variance and normally distributed data were assessed visually and then formally by the Shapiro–Wilk test. The condition factor based on Fulton's condition index as follows: CF =wet weight/ (shell length)^3 × 100. Images were taken with previously cited microscopes and software in Chapter 2, Section 2.2.3, page 43.

5.3. RESULTS

5.3.2.1 Morphometrics of subtidal mussels in field survey

Morphometrics of *Mytilus* spp., sex ratio and sample sizes of subtidal populations sampled are summarised in Table 1. Subtidal *Mytilus* spp., sampled from the North Buoy in Lough Hyne were larger than those sampled in Tranabo ($F=8.37$, $df=1$, $P<0.0001$). No clear seasonal pattern in spawning was detected in either population (Fig. 4).

Table 2. Morphometrics of *Mytilus* spp. populations sampled in Lough Hyne North Buoy and Tranabo Buoy in the form $\bar{x}$ (±SD). CI represents condition index. F:M:U represents the ratio of *Mytilus* spp. with female, male or undifferentiated gonads within the sample screened.

Location	Average Length (mm)	Condition Index	F:M:U	n
Cuanmore pier	61.10 (±11.85)	0.01 (±0.02)	0.37:0.60:0.03	30
North Buoy Lough Hyne	54.35 (±11.79)	0.04 (±0.36)	0.35:0.37:0.27	365
Tranabo	51.10 (±9.33)	0.04 (±0.42)	0.47:0.38:0.15	363

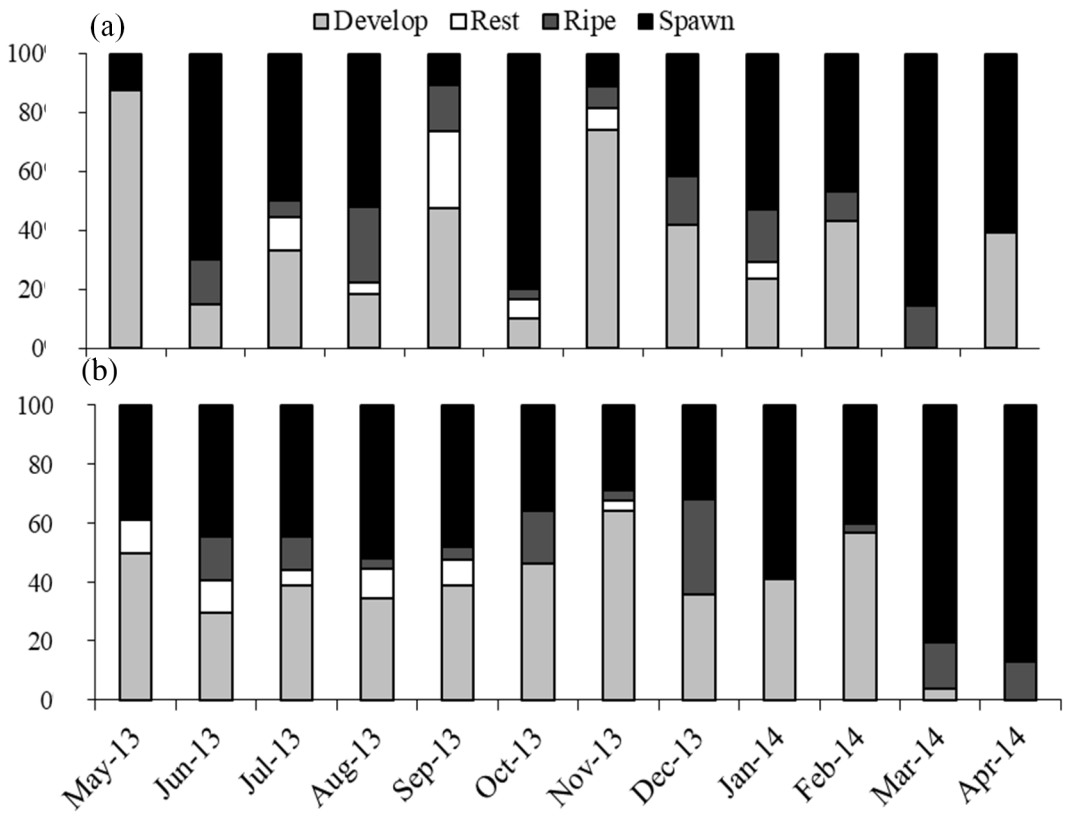

Fig. 4. The percentage of *Mytilus* spp. at each stage of gametogenesis from samples taken from (a) the North Buoy in Lough Hyne and (b) Tranabo bay

5.3.2.2 Histological screening for parasite taxa in field survey

Nine parasite taxa were recorded during the histological study, comprising of four macroparasite and five microparasite taxa. The apicomplexans gregarines were the most common parasite taxa (Table 3). Apicomplexans gregarines were followed in abundance by ciliates, prokaryotic inclusions (PIB), *Rickettsia*-like

organism/*Chlamydia*-like organism (RLO/CLO), trematodes, nematodes, coccidian and a cestode (Table 3).

Table 3. Overall parasite prevalence per site sampled and range (min-max) of intensity in brackets for subtidal populations of *Mytilus* spp. *Rickettsia*-like organism/*Chlamydia*-like organism and prokaryotic inclusion body are represented by RLO/CLO and PIB, respectively.

Parasite taxa	North Buoy Lough Hyne	Tranabo	Cuanmore pier
MICROPARASITE			
Ciliate	8.52 (1-121)	18.73 (1-31)	16.70 (1-2)
Coccidian	1.00 (1)	8.82 (1)	0.00
Gregarine	13.28 (1-124)	31.40 (1-85)	0.00
PIB	10.78 (1-44)	14.33 (1-78)	6.70 (1)
RLO/CLO	9.52 (1-48)	13.50 (1-14)	10.00 (1-4)
MACROPARASITE			
Cestode	0.25 (1)	0.00	0.00
Copepod	4.01 (1-4)	12.5 (1-3)	3.30 (1)
Nematode	7.02(1-2)	9.37 (1-2)	0.00
Trematode	9.52 (1-11)	11.85 (1-7)	3.30 (1)

The prevalence of parasite taxa fluctuated over the sampling period (see Fig. 5). *Nematopsis* sp., Schneider, 1892, oocysts (Fig. 6e) were the predominant gregarine infection, the most common parasites at all sites and months sampled. *Nematopsis* sp. were the only parasite taxon to significantly differ between with higher prevalence observed in Tranabo (χ^2=15.10, df=1, P<0.001). Ciliates were next most abundant taxon and were commonly found on gill filaments. Trematode metacercariae were more common than sporocyst infections. Infection usually occurred within digestive tubules. RLO/CLOs ranged from 1-48 in *Mytilus* spp. and were found predominately in the gills. Higher intensity of RLO/CLOs were seen in males (χ^2 =5.1, df=2, P=0.05). Copepods were mainly found in the digestive tract and to a lesser extent in the gills. It

is likely that *Mytilicola intestinalis*, Steuer, 1902, was the predominant copepod found in the digestive tract. This is due to the location and size of sections of copepod, however characteristics of *M. intestinalis* used to distinguish from the invasive *Mytilicola orientalis* Mori, 1935 such as were not apparent in a cross section. No difference was detected between sexes of *Mytilus* spp. and infection by parasite taxa.

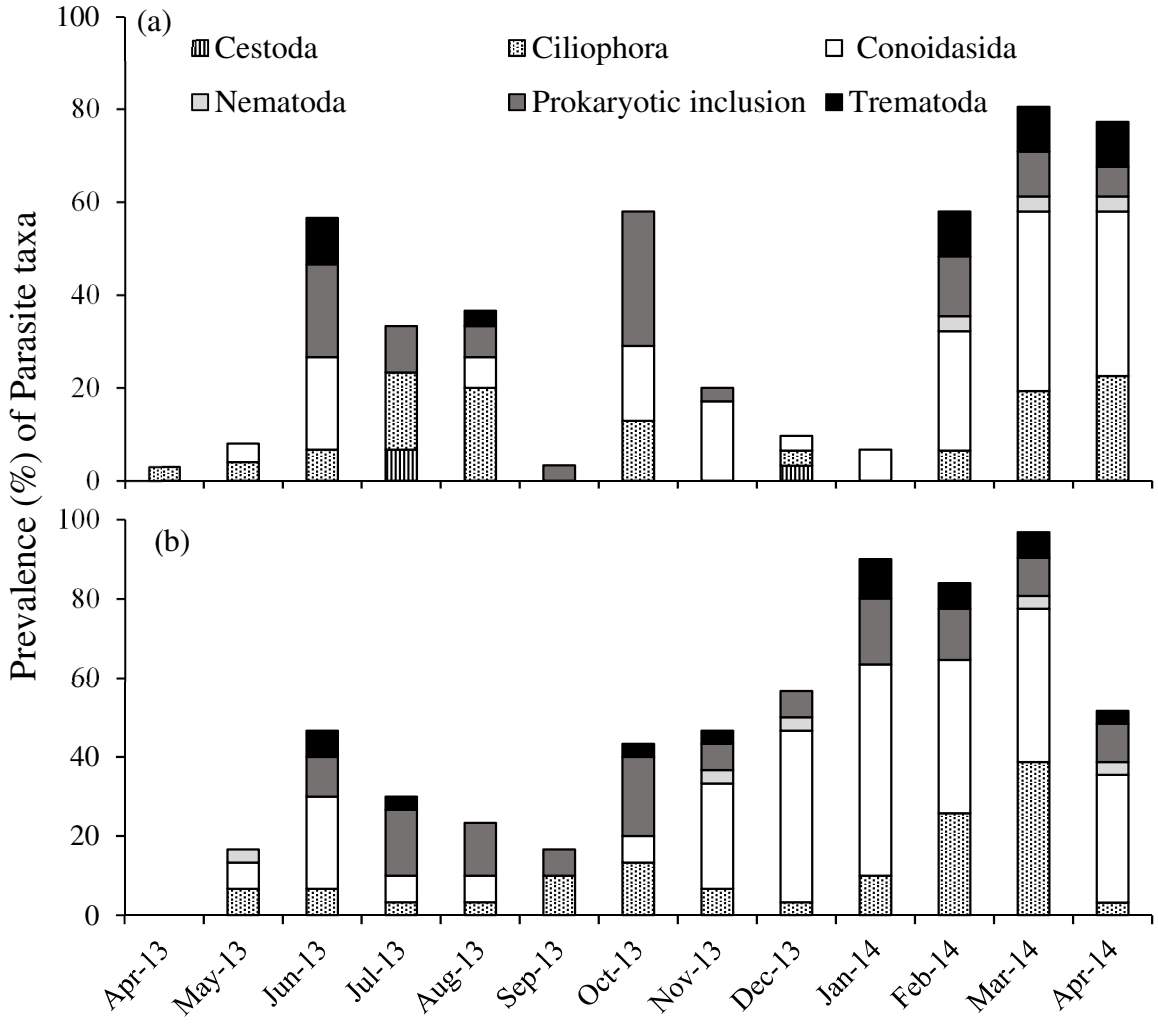

Fig. 5. Parasite prevalence for subtidal populations of *Mytilus* spp. sampled from a buoy in (a) Lough Hyne and (b) Tranabo bay from April 2013 until April 2014.

Coinfection, differing parasite taxa within a host, commonly occurred in screened *Mytilus* spp. Overall, coinfection was recorded in 21% of all *Mytilus* spp. and was significantly more common in *Mytilus* spp. populations in Tranabo bay (27%) than Lough Hyne (15%) (χ^2=15.48, df=1, P<0.0001). Coinfection was predominately between ciliates and other microparasite taxa for both locations (Table 4). Of the significant correlations between parasite taxa, predominantly negative correlations were found between prokaryotes and helminths.

Table 4. Overall correlations of taxa with other parasite taxa recorded in subtidal populations of *Mytilus* spp. sampled. *Rickettsia*-like organism/*Chlamydia*-like organism and prokaryotic inclusion body are represented by RLO/CLO and PIB, respectively. Asterisk denotes statistical significance: * for P<0.05, ** for <0.001 and *** for <0.0001. [1] represents helminth taxa

Parasite	Co-occurring Parasite	Correlation coefficient	Overall prevalence of coinfection
Ciliate	Cestode [1]	0.00	0.26
	Copepod	-0.01	0.66
	Gregarine	0.07***	3.95
	Nematode [1]	0.00	0.39
	PIB	0.01	2.50
	Trematode [1]	0.00	2.63
	RLO/CLO	0.01	3.16
Copepod	Cestode [1]	0.06	0.13
	Gregarine	0.40	1.97
	Nematode [1]	0.11***	0.26
	Trematode [1]	0.01	0.39
	RLO/CLO	0.02	0.92
	PIB	-0.01	0.00
Gregarine	Cestode [1]	-0.01	0.13
	Nematode [1]	-0.01	0.39
	Trematode [1]	-0.20***	1.57
	PIB	-0.14	1.18
	RLO/CLO	0.07	1.97
Nematode1	Trematode [1]	0.01	0.39
PIB	Trematode [1]	-0.33***	0.52

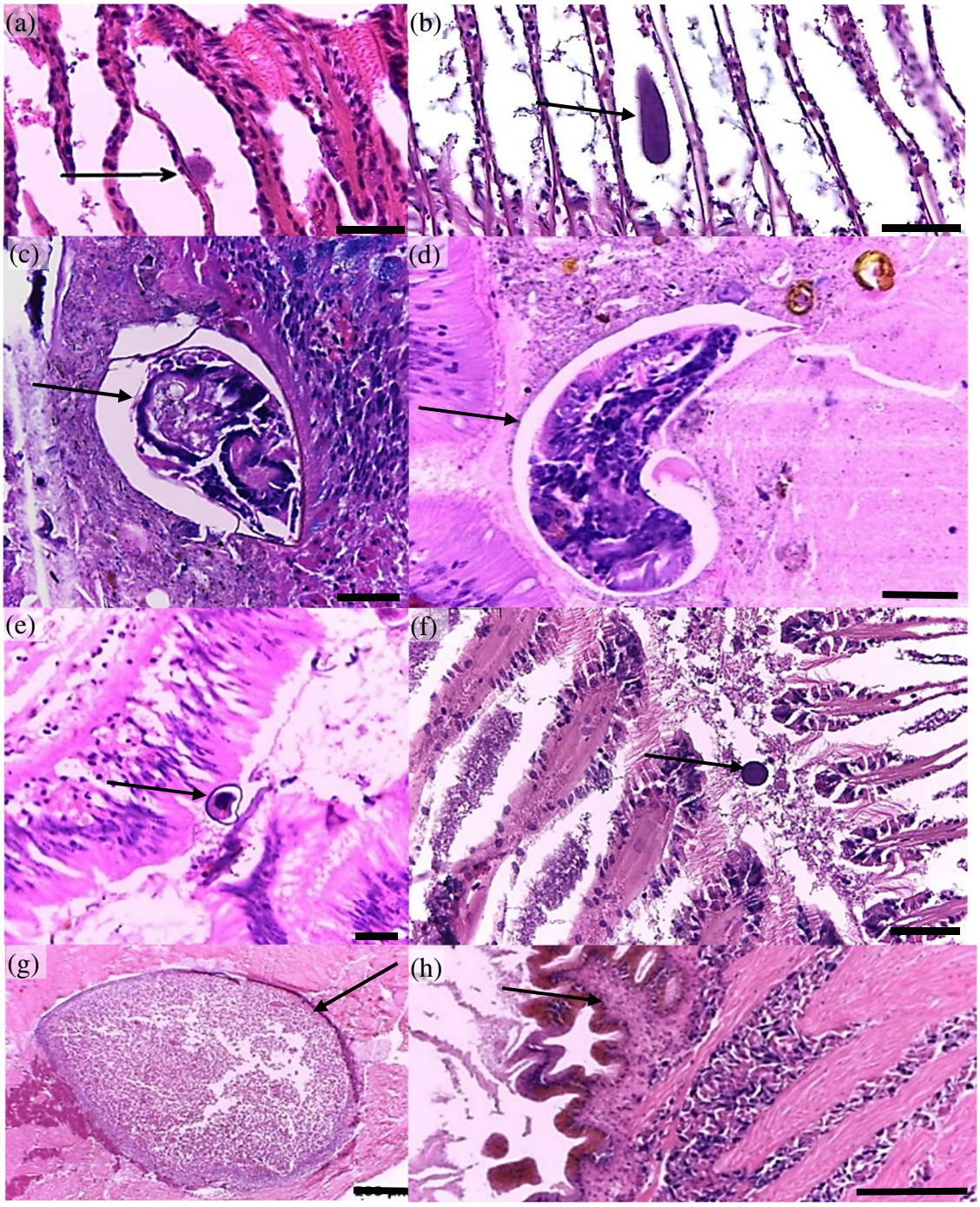

Fig. 6 Photomicrographs of parasites recorded in subtidal populations of *Mytilus* spp. sampled in Lough Hyne and Tranabo from April 2013 until April 2014. (a) *Rickettsia*-like organism/ *Chlamydia*-like organism on the gills (RLO/CLO), (b) Ciliate in gill tissue (c) Copepod in digestive tract, (d) Trematode metacercariae in digestive tubule (e) *Nematopsis* oocyst in the connective tissue, (f) Prokaryotic inclusion body (PIB) in gill tissue, (g) Granulocytoma in foot tissue & (h) Lipofuscin accumulation in mantle. (a)-(f) scale bars represent 20µm, 200µm for (g) and 100µm for (h). Haematoxylin and eosin stain.

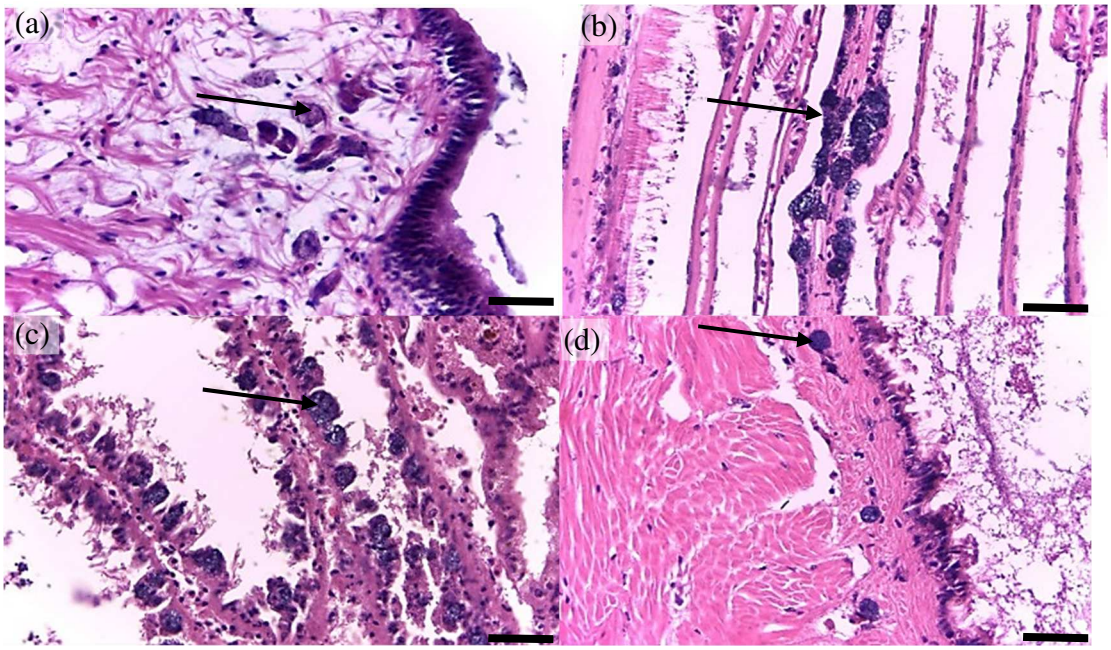

Fig. 7. Photomicrographs of haplosporidian-like particles recorded in (a) connective tissue, (b) gill tissue, (c) kidney tissue and (d) mantle tissues of subtidal *Mytilus* spp. sampled in Lough Hyne and Tranabo from April 2013 until April 2014. Scale bars represent 200µм for (a) and 20µm for (b), (c) and (d). Haematoxylin and eosin stain.

5.3.2.3 Observed pathological conditions in mussels in field survey

Four pathologies were recorded during the study; lipofuscin accumulation, inflammation, granulocytoma and pearl formation. Lipofuscin accumulation was the most common pathology of study and was recorded in just under half of all *Mytilus* spp. examined (Table 5) (Fig. 6(h)). The occurrence of the remaining pathologies did not differ significantly between sites or months sampled.

Table 5. Overall pathology occurrence and correlation with parasite taxa recorded in subtidal populations of *Mytilus* spp. sampled. *Rickettsia*-like organism/*Chlamydia*-like organism is represented by RLO/CLO. Asterisk denotes statistical significance: * for P<0.05, ** for <0.001 and *** for <0.0001.

Pathology	Prevalence	Parasite taxa	Correlation coefficient
Granulocytoma	4.72	Ciliate	-0.52***
		RLO/CLO	-0.45***
		Trematode	-0.50***
		Copepod	-0.29***
		Gregarine	-0.24***
		Lipofuscin	0.11**
Inflammation	11.55	Copepod	-0.31***
		RLO/CLO	-0.66***
		Trematode	-0.24***
		Ciliate	-0.13
Lipofuscin	47.77	Copepod	0.35***
		Trematode	-0.26***
		RLO/CLO	-0.13**
		Ciliate	-0.07*
Pearl formation	0.79		

5.3.3.1 Morphometrics of mussels in laboratory trials

Overall, 350 *Mytilus* spp. were screened during the study, 230 of which were included in the trials T1-3. In all three trials, no mortality was observed.

Table 6. Morphometric data of *Mytilus* spp. included in the trials carried out in October 2015. CI represents Fulton's Condition Index. Data are presented in the form of $\bar{x}$ (±SD)

Trial	n	Shell length (mm)	Whole Weight (g)	CI
1	54	42.30 (±2.89)	12.4 (±2.38)	0.01 (±<0.01)
2	48	34.06 (±8.12)	6.27 (±3.73)	0.01 (±<0.01)
3	128	41.86 (±2.17)	11.78 (±2.30)	0.03 (±0.32)
Total	230	40.48 (±5.40)	10.91 (±3.60)	0.02 (±0.24)

T 1 Intertidal and subtidal *Mytilus* spp. trial

Mytilus spp. in the laboratory trial were significantly larger in the intertidal than the subtidal habitat ($F=12.32$, $df=1$, $P<0.001$). *Mytilus* spp. morphometrics did not significantly vary within treatment groups between experimental runs.

T 2 Harmful algal bloom

Two *Mytilus* spp. spawned in both the second and third experimental run of T2. The individuals were still included in the analysis as their mantle retracted or shell closed in response to mechanical stimulation. *Mytilus* spp. morphometrics did not vary within treatment groups, between experimental runs or between treatment groups.

T 3 Acute exposure nutrient enrichment and host susceptibility trial

Mytilus spp. morphometrics did not significantly vary within treatment groups, between experimental runs or between treatment groups.

Parasite taxa detected in mussels screened prior in laboratory trials

Macroparasite taxa from Phylum Platyhelminthes and Copepoda were recorded in *Mytilus* spp. Trematode taxa were the most abundance parasite taxa recorded. In addition to natural infections of *E. patellae*, four other trematode taxa; *Renicola* spp., *Microphallus* spp., *Himasthla* spp, and *Proctoeces maculates* (Looss, 1901); were recorded in *Mytilus* spp. included in T1-3 (see Table 7). *Renicola* spp. was the most common taxa harboured by *Mytilus* spp. for all laboratory trials. The copepod *M. intestinalis* and the turbellarian *Urastoma cyprinae* (Graff, 1882) were also recorded

in *Mytilus* spp. (Table 7 & 8). Negative controls of each trial, demonstrate the differences in natural infections between *Mytilus* spp. populations sampled (Table 7 & 8).

Table 7 Prevalence of trematode taxa in populations of *Mytilus* spp. included in the three trials run in October 2015. Data are presented in the form of prevalence (min-max). – or + indicate whether *Mytilus* spp. was exposed to *Echinostephilla patellae* cercariae. For Trial 1, I represent intertidal and S subtidal *Mytilus* spp. collected in Lough Hyne. For trial 2 Cu represents Cusheen, LH Lough Hyne, R Ringaskiddy. For trial 3 C represents the control, L low nutrient enrichment, M medium enrichment, and H high enrichment treatments.

Trial	Treatment	N	*Echinostephilla patellae*	Renicola spp.	Microphallus spp.	Himasthla spp.	Proctoeces maculatus
1	I-	12	91.67 (1-198)	75.00 (1-142)	0.00	0.00	0.00
1	I+	12	75.00 (2-128)	33.33 (1-3)	0.00	8.33 (1-3)	0.00
1	S-	12	16.67 (7-25)	0.00	0.00	8.33 (52)	0.00
1	S+	12	33.33 (1-6)	0.00	0.00	0.00	0.00
2	Cu-	9	33.33 (1-3)	77.78 (3-80)	0.00	88.89 (2-26)	0.00
2	Cu+	9	22.22 (1)	66.67 (3-40)	0.00	77.78 (2-20)	11.11
2	LH-	9	22.22 (1)	55.56 (1-2)	0.00	44.44 (4-38)	0.00
2	LH+	9	55.56 (1-14)	44.44 (1-3)	0.00	1.00 (1-7)	0.00
2	R-	9	22.22 (1)	66.67 (1-2)	0.00	11.11 (4)	0.00
2	R+	9	33.33 (1)	0.00	0.00	33.33 (2-18)	0.00
3	C-	17	5.88 (1)	100 (2-129)	5.88 (1)	0.00	0.00
3	C+	16	6.25 (1)	93.75 (2-231)	0.00	0.00	0.00
3	L-	14	14.29 (1-3)	92.86 (1-94)	0.00	0.00	0.00
3	L+	17	5.88 (3)	100 (3-22)	0.00	11.76 (4-38)	0.00
3	M-	16	12.50 (5)	100 (2-105)	0.00	0.00	0.00
3	M+	16	12.50 (1)	100 (4-177)	6.25 (1)	6.25 (1-7)	0.00
3	H-	15	6.67 (1)	86.67 (3-70)	0.00	13.33 (2-4)	0.00
3	H+	17	11.76 (1-5)	100 (6-208)	0.00	5.88 (1)	0.00
Grand Total		230	23.10 (1-198)	73.91 (1-231)	0.87 (1)	17.39 (1-52)	0.43

Table 8 Prevalence of macroparasites other than trematodes and pearl formation from populations of *Mytilus* spp. included in the three trials run in October 2015. – or + indicate whether *Mytilus* spp. was exposed to *Echinostephilla patellae* cercariae. For Trial 1, I represent intertidal and S subtidal *Mytilus* spp. collected in Lough Hyne. For Trial 2, Cu represents Cusheen, LH Lough Hyne, R Ringaskiddy. For Trial 3, *Mytilus* spp. collected from Blackrock were assigned to the control; C, low nutrient enrichment; L, medium enrichment; M, or high enrichment treatments; H.

Trial	Treatment	n	Pearl formation	*Urastoma cyprinae*	*Mytilicola intestinalis*
1	I-	12	0.00	0.00	8.33
1	I+	12	0.00	0.00	33.33
1	S-	12	0.00	0.00	0.00
1	S+	12	0.00	0.00	0.00
2	Cu-	9	0.00	0.00	33.33
2	Cu+	9	0.00	0.00	0.00
2	LH-	9	0.00	0.00	0.00
2	LH+	9	0.00	0.00	0.00
2	R-	9	0.00	0.00	0.00
2	R+	9	0.00	0.00	0.00
3	C-	17	5.88	0.00	0.00
3	C+	16	6.25	0.00	0.00
3	L-	14	21.43	0.00	0.00
3	L+	17	23.53	0.00	0.00
3	M-	16	25.00	0.00	0.00
3	M+	16	18.75	0.00	0.00
3	H-	15	6.67	6.67	0.00
3	H+	17	23.53	0.00	0.00
Grand Total		230	9.13	0.43	2.17

For T1 intertidal (n=10) and subtidal (n=10) *Mytilus* spp. were screened prior to laboratory trials. Three parasite taxa were observed, *Himasthla* spp., *Renicola* spp. and *M. intestinalis*. Prevalences of all parasite taxa were higher in *Mytilus* spp. from the intertidal of Lough Hyne; 100% of screened *Mytilus* spp. harboured *Himasthla* spp., 60% harboured *Renicola* spp. and 10% contained *M. intestinalis*. In the subtidal *Mytilus* spp. screened, *Renicola* spp. was the only taxa and a prevalence at 10% was observed. For T2, intertidal *Mytilus* spp. from Cusheen, Lough Hyne and Ringaskiddy were screened prior to the laboratory trial. Prevalences of *Renicola* spp. in *Mytilus* spp. from Cusheen were observed at 83% and 40% in both Lough Hyne and Ringaskiddy. Higher prevalence of *M. intestinalis* was observed in *Mytilus* spp. from Ringaskiddy (50%) relative to Cusheen (25%). The copepod was absent from *Mytilus* spp. screened from Lough Hyne. In T3, *E. patellae* (12%), *Renicola* spp. (34%), the *Proctoeces maculatus* (Looss, 1901) (1%), copepods (2%) and pearls (18%) were observed in *Mytilus* spp. (n=100) sampled from Blackrock.

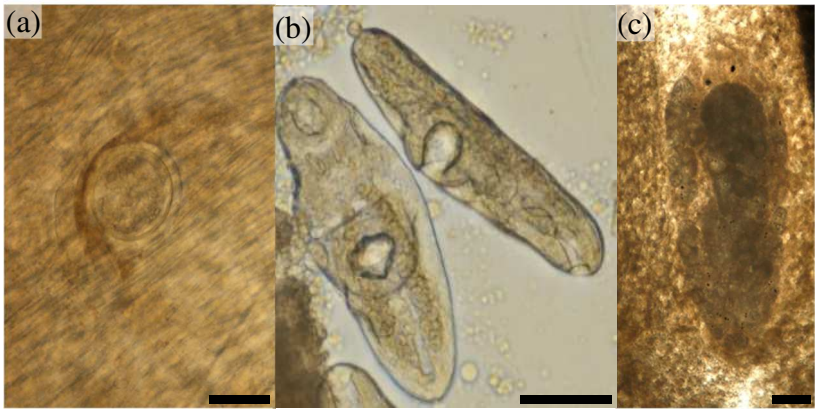

Fig. 8 Photomicrographs of helminth taxa recorded during the *Mytilus* spp. trials carried out in October 2015 (a) *Echniostephilla patellae* metacercariae in the bivalve *Mytilus* spp. (b) *Proctoeces maculatus* cercariae (c) Turbellarian *Urastoma cyprinae*. Scale bars indicate 100µm.

T 1 Intertidal and subtidal *Mytilus* spp. trial

Prevalence and intensity of *E. patellae* did not significantly differ between treatment groups. *Echinostephilla patellae* was significantly more prevalent in intertidal *Mytilus* spp. relative to subtidal *Mytilus* spp. controls (χ^2 =10.74, df=1, P<0.00) (Table 7). Prevalence of *Renicola* spp. and the copepod *M. intestinalis* were higher in intertidal *Mytilus* spp. than subtidal specimens (Table 7). Negative controls of intertidal and subtidal *Mytilus* spp. had similar prevalences and intensities of trematode taxa and copepods (Table 7).

T 2 Harmful algal bloom

Prevalence and intensity of *E. patellae* did not significantly differ between treatment groups. Prevalence *Renicola* spp. (χ^2 =7.65, df=2, P<0.05) and *Himasthla* spp. (χ^2=8.25, df=2, P<0.05) significantly differed between controls. Primary intermediate infection by *Proctoeces maculatus* was observed in a negative control of *Mytilus* spp. from Cusheen. Higher prevalences of both trematode taxa were observed in *Mytilus* spp. sampled from Cusheen (Table 7).

T 3 Acute exposure nutrient enrichment and host susceptibility trial

Echinostephilla patellae prevalence and intensity did not differ between controls or within treatments of differing experimental runs. Prevalence and intensity of *E. patellae* also did not significantly differ between different treatment groups. The turbellarian *Urastoma cyprinae* was recorded in one specimen in the negative control of the high treatment. *Renicola* spp. and the pathology of pearl formation were more common in *Mytilus* spp. sampled from Blackrock (Table 7 & 8). *Renicola* spp.

prevalence and intensity were high in all enrichment treatments and did not statistically differ. Differences in the occurrence of pearl in treatments were not statistically significant, despite seeming more common in *Mytilus* spp. exposed to moderately enriched conditions (Table 8).

Overall, coinfection of parasite taxa in *Mytilus* spp. included in all trials was low (1.42%) (Fig. 9). In T3 coinfection was the highest of all the three trials (Fig. 9). Recorded coinfections of macroparasites were predominantly in the form of trematodes with copepods. Coinfections of trematode taxa were in the form of *E.*

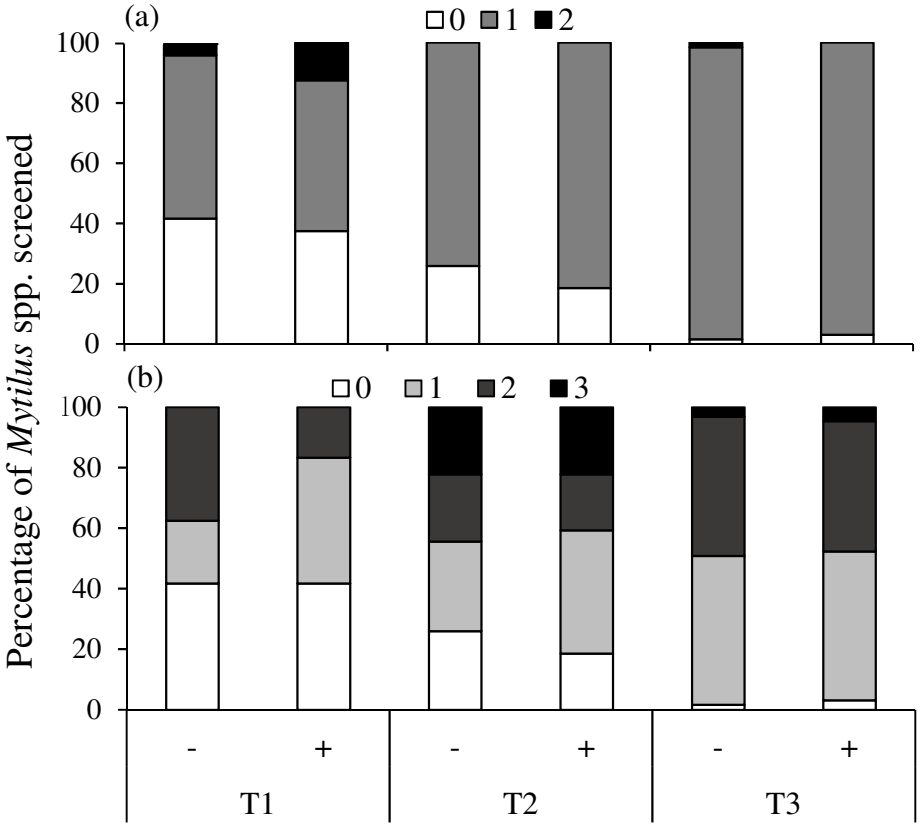

Fig. 9 (a) The prevalence of *Mytilus* spp. with no macroparasite taxon (0), harbouring a single parasite taxon (1) or two parasite taxa (2). (b) The prevalence of *Mytilus* spp. harbouring no trematode taxon (0), one trematode taxon (1) or multiple trematode taxa (2, 3). Exposure to *Echinostephilla patellae* cercariae is represented with "+" and controls, to which no cercariae were added, are represented with "-".

patellae, *Himasthla* spp. and *Renicola* spp. Coinfections between *E. patellae* and other trematode metacercariae were positively correlated for T2 and T3. The intensity of *Himasthla* spp. and *E. patellae* in the foot of *Mytilus* spp. were negatively correlated, however it was statistically insignificant ($r=-0.24$, $df=14$, $P>0.05$).

5.4. DISCUSSION

Both the field and laboratory trials recorded a range of largely non-pathogenic parasite taxa in *Mytilus* spp. In the field study, parasite communities or pathologies did not differ between subtidal populations in sites of differing water quality except for a higher occurrence of gregarines in Tranabo bay and a single cestode observed in the North Buoy population. The mooring lines sampled in Lough Hyne and Tranabo are connected by water at high tide. and so subtidal populations of *Mytilus* spp. sampled may have experienced similar exposure to contaminants and parasite taxa. The study would need additional replicates of subtidal *Mytilus* spp. populations to investigate the potential effect of surrounding water quality and parasite communities. An alternative study to address the hypothesis would be a transplant experiment of samples from a common subtidal *Mytilus* spp. population for further investigation into the effect of water quality on parasite communities.

Parasite communities of *Mytilus* spp., screened in the field study were dominated by apicomplexan gregarines, followed by ciliates, prokaryotic inclusions (PIB), *Rickettsia*-like organism/*Chlamydia*-like organism (RLO/CLO), trematodes, nematodes, a coccidian and a cestode. In relation to seasonality of parasites this study agrees with work in UK estuaries (Bignell *et al.,* 2008). Except for the pathology of lipofuscin accumulation, no trends in prevalence of parasites or intensity in relation to month or season were found. Similar patterns have been reported in *M. galloprovincialis* with peaks of lipofuscin accumulation recorded in summer month (Koukouzika *et al.,* 2009). Parasite composition differed slightly from a previous health survey of cultured and wild *Mytilus* spp. (Lynch *et al.,* 2014). In the present study, copepods were the dominant macroparasites followed by trematodes while Lynch *et al.,* (2014) found the reverse. Parasite communities' composition agree with that in the Black Sea and Netherlands (Lauckner 1983).

The field study, recorded lower parasite prevalence and higher intensity extent of infections and pathologies than previous work on *Mytilus* spp. in Irish marine waters (Lynch *et al.,* 2014, Giltrap *et al.,* 2016). This observation could be due to subtidal habitat and immunological vigour of *Mytilus* spp. screened. The fact that *Mytilus* spp. in the field study were sampled from the subtidal zone could explain the low macroparasite prevalences recorded (Buck *et al.,* 2005, Brenner *et al.,* 2014). Although, intertidal molluscs are key hosts for digenean trematodes and other macroparasite taxa, increased distance between potential hosts and current hosts lowers the likelihood of a macroparasite encountering and infecting a potential host (Galaktionov *et al.,* 2003, Buck *et al.,* 2005, Brenner *et al.,* 2014). This is likely the case for trematodes typical intermediate hosts may be restricted to nearshore habitats e.g. *Littorina* spp. abundant in mid- and low intertidal zones but is rare in shallow subtidal habitats (Reid 1996, Carlson *et al.,* 2006). Thus, the further the populations of *Mytilus* spp. are from the substrate or intertidal zone, the lower their encounter rate with preceding trematode hosts and subsequent infection rates would be. In the German Bight, offshore mussels were not infected by trematodes or shell-boring polychaetes(Buck *et al.,* 2005, Brenner *et al.,* 2014). Low trematode prevalence in this study was recorded in subtidal *Mytilus* spp. sampled for laboratory trials the same location which were sampled simultaneously. Higher prevalence and intensity of trematodes were recorded in intertidal *Mytilus* spp. in Lough Hyne intertidal relative to subtidal *Mytilus* spp. sampled at the same time. *Mytilus edulis* haemocytes have been found to be more active in phagocytosis and superoxide generation relative to sympatric bivalves the edible cockle *Cerastoderma edule* and the razor-shell *Ensis siliqua* (Linnaeus, 1758) (Wootton *et al.,* 2003).

By definition a parasite is an organism that exerts some harm on the host (Thieltges *et al.,* 2013). The extent of harm varies between taxa and life stages and a well-adapted parasite can be intermediately pathogenic or non-pathogenic (Combes, 1997). The pathology of parasite taxa recorded in the field study, trematodes and the turbellarian

U. cyprinae, is dependent on the parasitic life stage and intensity of infection (Lauckner, 1983, Thieltges, 2006, Stier *&* Thieltges 2015). Trematode sporocyst infections can be highly pathogenic as heavy infections can result in parasitic castration, weakness in valve closure, reduced byssal thread activity and may affect growth (Matthews 1973, Lauckner ,1983, Coustau *et al.,* 1991; 1993, Thieltges 2006, Stier *&* Thieltges, 2015). The low prevalence of trematode sporocysts in the subtidal specimens in Lough Hyne and of *Proctoeces maculatus* in the intertidal specimens on the east coast suggest that trematode primary infection is not a major threat to the health of either population sampled.

The histological study found no association between local inflammation or pathology and recorded parasite taxa. Gregarines in the form of *Nematopsis* sp. oocysts were the most prevalent parasites for all of the months and both sites sampled. Gregarine infection has been recorded to cause focal and benign inflammation in *Mytilus* spp. (Bower *et al.,* 1994). Ciliates were another highly prevalent microparasite. Ciliates are commensals but can become pathogenic in high intensities or if the host is stressed (Lauckner 1983). The copepod *M. intestinalis* was the most prevalent macroparasite but is also considered to be a commensal (Francisco *et al.,* 2010). The pathology of the copepod is low and generally restricted to mechanical damage of tissue around the site of its hook-like appendages (Moore *et al.,* 1978). A 10-year study in the UK failed to establish *M. intestinalis* as a determining or major contributing factor in bivalve mortality (Davey & Gee 1976). RLO/CLOs have been recorded in a wide range of bivalves and are usually non-pathogenic (Lauckner 1983).

Tissue compression techniques, utilised in the laboratory trials, recorded a smaller range of parasite taxa then histological techniques. In this study, no difference in the prevalence or intensity of the trematode *E. patellae* was recorded during the trials. Although, not statistically significant there seemed to be a general trend of increased *E. patellae* prevalence in *Mytilus* spp. exposed to cercariae. Coinfection of trematode

taxa was common in laboratory trials T2 and T3. The high level of natural trematode infection in the foot tissue of *Mytilus* spp. could have impaired *E. patellae* infection through competitive displacement. *Echinostephilla patellae* cercariae may have been prevented from encysting in the foot by metacercariae as the area was already occupied by *Renicola* spp. and *Himasthla* spp. Pearls were recorded predominantly in the Blackrock population. The formation of such structures in bivalves have been associated with parasitic trematodes, copepods, cestodes, nematopsis oocysts and inorganic irritants (Garner 1872, Sindermann & Rosenfield 1967 Dakin, 1913). In particular previous work on the south coast of Ireland have documented the formation of pearls around metacercariae of *Gymnophallus* spp. in the bivalves *Mytilus* spp. and *Cerastoderma edule* (Fermer *et al.,* 2011, Morgan 2013). However relative to other studies the prevalence and intensities of pearls in *Mytilus* spp. was lower (94%) have been recorded with up to 264 recorded in a single *Mytilus edulis* (Fernandes & Seed 1983, Ambariyanto & Seed 1991).

When the life cycle of *E. patellae* was initially investigated by Kollien (1996), *Mytilus* spp. was not considered in the list of secondary hosts, the gastropod *P. vulgata* was investigated but was exposed to unnaturally high doses of *E. patellae* cercariae. Prinz *et al.,* (2010b) exposed *Mytilus* spp. to varying doses of *E. patellae* and the trematode successfully infected the bivalve's foot. The low doses of 10 cercariae per mussel could be a contributing factor to the failure to detect a statistically significant difference in *E. patellae* prevalence and intensity between *Mytilus* spp. in the trials. In previous work the doses were successful and are likely more biologically realistic (Poulin 2010, Prinz*et al..,* 2010b).

Biomass of *Mytilus* spp. was unlikely a determining factor for the differences in parasite prevalences observed in the laboratory trials. The only difference in size between *Mytilus* spp. specimens was recorded in T1, where larger specimens were sampled from intertidal relative to the subtidal zone of Lough Hyne. This difference

is likely due to sampling methodology, as while intertidal and subtidal differ in size due to their differing growth rates, typically larger specimens are recorded in the subtidal relative to the intertidal zone (Seed, 1969, Page & Hubbard 1987). Subtidal *Mytilus* spp. can grow continuously while intertidal *Mytilus* spp. undergo periods of exposure to air and suppressed feeding activity (Labarta *et al.,* 1997, Tagliarolo *et al.,* 2012). While the size of *Mytilus* spp. is not necessarily related to their age, larger *Mytilus* spp. are likely to have a higher encounter rate with and infection by parasites (Buschbaum & Saier, 2001, Nikolaev *et al.,* 2006). It is acknowledged that there was a difference in holding time of *Mytilus* spp. between runs of laboratory trials. *Mytilus* spp. included in the second and third experimental run of the laboratory trials were held for 48 and 60 hours, respectively, more that individuals in the first run. This may have had an effect, but one which was undetected in parameters measured as there was no significant difference in *Mytilus* spp. mortality, size, spawning or parasite infection between experimental runs.

This study cannot conclusively report the susceptibility of *Mytilus* spp. to *E. patellae* cercariae exposed to environmental stressors simulated or otherwise. The interpretation of results was confounded by natural infection of *Mytilus* spp. Future work, could include either farmed or subtidal *Mytilus* spp. in laboratory trials along with work to investigate the feasibility of estimating the age of *E. patellae* metacercariae according to its morphology. This would allow for the differentiation between pre-existing natural *E. patellae* and experimental infections. The effect of environmental stressor eutrophication on host: parasite dynamics requires further work. The susceptibility of *Mytilus* spp. to parasite taxa in eutrophic conditions is particularly necessary as suspended mussel aquaculture has been proposed as one possible solution to mitigate coastal water quality problems associated with excess nutrients (Edebo *et al.,* 2000, Gren *et al.,* 2009, Lindahl *et al.,* 2005). There is a need to evaluate the risk that parasites taxa recorded in both studies, may pose to *Mytilus* spp. under climate change.

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CHAPTER 6: General Discussion

To assess an impacted ecosystem, knowledge of pathological conditions and parasites is essential, as it provides valuable insight into the health of host populations and the state of the ecosystem. Data on dominant parasites in multiple host taxa can inform on how parasites and hosts respond to current anthropogenic impacts and aid in the creation of accurate predictions of e.g. a changing climate, increased anthropogenic impact on a specific site in marine waters.

The current study was undertaken to gain knowledge on how the distribution of parasite communities of wild invertebrate taxa on the southwest coast of Ireland varied in response to ecosystem quality. The focus on larval trematodes developed as the taxa are abundant in the intertidal zone and have been proposed, by multiple reviews, as indicators of ecosystem health (Lafferty 1997, Mouritsen & Poulin 2002, Huspeni *et al.,* 2005, Marcogliese 2005, Vidal- *et al.,* 2010, Prinz *et al.,* 2010, Sures *et al.,* 2017).

The observed variation of parasite and trematode communities in relation to ecosystem quality appeared dependent on site specific local factors, host taxa screened, parasite taxa and the definition of quality considered. Ecosystem quality was defined by policy directive classifications in Chapters 2 & 4, and by statutory protection in Chapters 3 & 5. In Chapter 2, metacercariae of the dominant trematodes *Echinostephilla patellae* and *Renicola* spp. were also more prevalent in the site of higher WFD status quality. Trematode primary infection has been suggested as an indicator for water quality (Huspeni *et al.,* 2005). Work in Northern Ireland found that *Cryptocotyle lingua* cercariae in *L. littorea* were negatively affected by poor water quality and had impaired swimming and potentially transmission (Cross *et al.,* 2001; 2003; 2005). In Chapter 2 the prevalence of primary infections in *Littorina littorea* did not differ

between sites but the number of functional groups was higher in the site of good water quality. In Chapter 4, trematode prevalence, intensity and community richness were greater in the site of low water quality. In Chapter 3, parasite communities of *Patella vulgata* were similar within and outside the marine reserve. The heterogeneous distribution of the dominant trematode, *E. patellae*, within and outside of the marine reserve was proposed to be determined by local site-specific factors, such as wave exposure and local currents rather than the level of protection. In Chapter 5, gregarines were the only parasite taxon harboured by subtidal *Mytilus* spp. that significantly differed between populations sampled as significantly higher prevalences recorded outside of the marine reserve. In Chapter 5, low prevalence of trematode taxa was recorded in both subtidal *Mytilus* spp. populations was likely to be due to their distance from avian vertebrate and intermediate invertebrate hosts. The lower prevalence of parasite taxa in subtidal *Mytilus* spp. relative to intertidal aquaculture stocks has already been documented (Buck *et al.,* 2005, Brenner *et al.,* 2012). Subtidal *Mytilus* spp. may not be very susceptible to recorded parasites under current environmental conditions. The effect abiotic environmetnal stressor in the form of nutrient enrichment was inconclusive for the *Mytilus* spp. trials due to the high levels of natural infections.

Data generated by this study contribute novel findings on the factors that influence the distribution and abundance of parasites within the intertidal environment of sites sampled. Chapters 2 and 3 addressed neglected aspects of parasite ecology in relation to marine conservation, EPA quality classifications and the effect of surrounding intertidal biota. At present, few conservation studies consider parasites (Nichols *et al.,* 2011, Gómez *et al.,* 2013). The literature review of Chapter 3 returned 12 studies on the topic of parasites in MPAs. Parasites may be facilitated by conservation and "jump" between hosts (Kermack & McKendrick, 1927, Lebarbenchon *et al.,* 2006).

Chapters 4 & 5 addressed the impact of eutrophication and habitat type on the host susceptibility. Findings from Chapters 2 and 4 suggest that trematode communities, observed, varied in relation to environmental stress. The variation was not consistent between environmental stressors considered or parasite taxa recorded. The magnitude of the effect of environmental stressors on a trematode taxon may vary within one between lifecycle stages and geographic regions (Marcogliese, 2016). Regional variation between has been documented for the trematode *Renicola* spp. utilising *L. littorea*, as a primary intermediate host. In the Barents Sea the optimum temperature range for the release of cercariae from *L. littorea* inhibited the release of cercariae of the same trematode and host taxa in the Wadden Sea (Thieltges *et al.,* 2006, Prokofiev *et al.,* 2016).

Previous parasitological work has illustrated the ubiquity of parasites in intertidal molluscs on the south coast of Ireland and the dominance of trematodes within the parasite communities (Prinz *et al.,* 2010, Fermer *et al.,* 2011). The study highlights the need to adopt a more a nuanced approach to host-parasite ecology. It is frequently reported that environmental stressors can impact water quality, affect the immunological response in aquatic biota and make them more susceptible to parasitic infection and increasing parasite prevalence (Khan and Thulin, 1991). The environmental stressor may also negatively affect the transmission of parasite taxa particularly if the taxa requires multiple hosts to complete its lifecycle (Pietrock & Marcogliese 2003). The response of parasites to environmental stressors is known to be varied and complex (Lafferty 1997, Blanar *et al.,* 2009, Vidal-Martinez *et al.,* 2010). Data from the study agree this the general findings of previous work and suggest that the distribution, prevalence and intensity of trematode taxa varied in response to environmental stressors in the form of nutrient enrichment. The effect of local biotic factors are likely context depenant as the significantly was not consistent between sites or host species sampled. In Chapter 2 the prevalence of *Echinostephilla*

patllae positively correlated with the density of its host *Patella vulgata* in the site of good water quality but not in the marine reserve.

This study attempted to provide robust baselines of trematode abundance and distributions in host species screened and sites sampled. The main technique of the study, tissue compression, provided a cost effective and conservative estimate of parasite abundance in invertebrates screened. In the detection of trematode taxa, tissue compression has been demonstrated to be equally effective or even outperform resource-heavy molecular techniques such as quantitative polymerase chain reaction (qPCR) (Markowitz *et al.,* 2016) or fluorescent tagging, respectively (LaFonte *et al.,* 2015). Tissue compression still provides a conservative estimate of trematode abundance as it is not able to detect early primary trematode infections (miracidia), and externally encysting metacercariae. Tissue compression was thus adopted to allow for a more cost effective and accurate estimate of trematode identity and abundance. Parasite identification was based on morphology, so cryptic species, which occur particularly in trematodes relative to other helminths, were grouped in a species complex (Leung *et al.,* 2009, Pérez-Ponce de León *et al.,* 2017). Larval trematodes infect specific host tissues preferentially e.g. palps or abductor muscles (Thieltges 2006, de Montaudouin *et al.,* 2009, Prinz *et al.,* 2010) and may be missed in the 1cm sampled from the of host for histological screening. Trematode taxa are difficult to accurately identify from histological slides without further molecular means.

There are several avenues future work could investigate from the current study. Work could consider the factors that influence the distribution and abundance of parasites within an environment and consider additional hosts other than molluscs when investigating the effect of environmental stressors on trematode prevalences and intensities (Thieltges &Wegner, 2016, Poulin *et al.,* 2016). The potential for protected areas and varying levels of eutrophication to facilitate trematode infection also

warrants further work. It is acknowledged that Lough Hyne may not be representative of the majority of MPAs. Unlike other MPAs, Lough Hyne is an extremely sheltered area and is well established as it was designated 36 years ago. The potential of MPAs to encourage parasite abundance within their boundaries should be evaluated on a larger spatial scale.

The potential of eutrophication to facilitate trematode abundance, should be investigated with manipulative laboratory trials conducted in tandem with field assessments of cercarial abundance in the water column. Manipulative field experiments in which the abundance of trematode is altered, e.g. exclusion from certain areas, are often logistically challenging (Sousa 1999, Kaplan *et al.,* 2009). Field surveys of trematode cercariae are also difficult, as cercariae are soft bodied, short lived and typically missed with traditional plankton survey techniques (Fingerut *et al.,* 2003, Zimmer *et al.,* 2009). However, estimations of cercariae in the water column have been done by screening specific hosts in the field and enumerating the amount of cercariae "shed" during timed intervals. After collection the invertebrate, was placed within a modified a container with several funnels than connect to a pump which water is pulled through, later filtered over filtered through a mesh (Fingerut *et al.,* 2003). Alternatively, a broader approach was undertaken in Californian estuaries where seawater was directly filtered through a mesh with a modified pump. Abundance of cercariae in estuaries was calculated by timed interval samples as a proxy for volume filtered (Zimmer *et al.,* 2009).

To predict the effects of current and potential environmental stressors on parasites, robust estimates of their abundance and response to biologically realistic conditions are needed. Predications on the response of parasite taxa to predicated changes have largely focused on the effect of temperature on a single trematode taxon (Poulin 2006, Macrogliese 2016). Thus, it could be postulated that accurate predictions of parasite

taxa's response to predicated climate change are difficult to make, due to the gaps in the knowledge, the inherent complexity of the host-parasite relationship and the neglect of environmental stressors outside of predicted anthropogenic warming (Harvell *et al.,* 1999, Lafferty & Ward 2004, Poulin, 2006, Marcogliese, 2016).

One such gap is the sublethal effects of parasites on intermediate hosts and the potential effect on surrounding biota. This is need in the case of the commonly parasitised taxa *P. vulgata* and *C. maenas* due to the high intensities of trematode metacercariae recorded and the ecological roles of the invertebrate hosts. Only primary intermediate infections of *E. patellae* in *P. vulgata* have been considered (Rees, 1934). Although, the effects of parasite taxa recorded within *P. vulgata* in Chapter 2 and 3 are largely considered non-pathogenic to the gastropod (Crewe, 1951, Lauckner, 1980) or have yet to be studied, as in the case of the cestode *O. insignis* (Crewe, 1947). The pathology of metacercariae is dependent on the intensity of infection (Fredensborg *et al.,* 2004, Thieltges, 2006).

Findings from the study thesis suggest that while trematode prevalence, intensity and distribution seemed to vary in relation to environmental stressors, further work should investigate the taxa's sensitivity to a range of environmental stressors prior to their use as potential bioindicators (Goodsell *et al.,* 2009).

There may be potential for trematodes as indicators for "Good Environmental Status" (GES) as defined by the Marine Strategy Framework Directive (MSFD). The food web descriptor (D4) in MSFD has been considered the productivity of key species or tropic groups, the proportion of selected species at the top of the food web and the abundance/ distribution of key trophic species (Rombouts *et al.,* 2013, Berg *et al.,* 2015). In particular, digenean trematodes require multiple hosts to complete their lifecycle, can be tropically transmitted and have been used to estimate the abundance

of final hosts (Byers *et al.,* 2011). However, at present, it is recommended that trematodes should be used in tandem with traditional approaches such as traditional biomarkers or communities' surveys. The reasons for the inclusion of trematodes are the taxa's recorded dominance in parasite communities, ubiquity and reported ability to affect host physiology and behaviour of invertebrate hosts (Lauckner, 1980; 1983, Theiltges *et al.,* 2006). This recommendation, is particularly relevant for studies that focus on the physiological responses of invertebrates as indicators of environmental stress or studies aiming to evaluate the effects of predicted climate change (Mouritsen *et al.,* 2002a, Kim *et al.,* 2008, Rodrigues *et al.,* 2014).

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APPENDIX

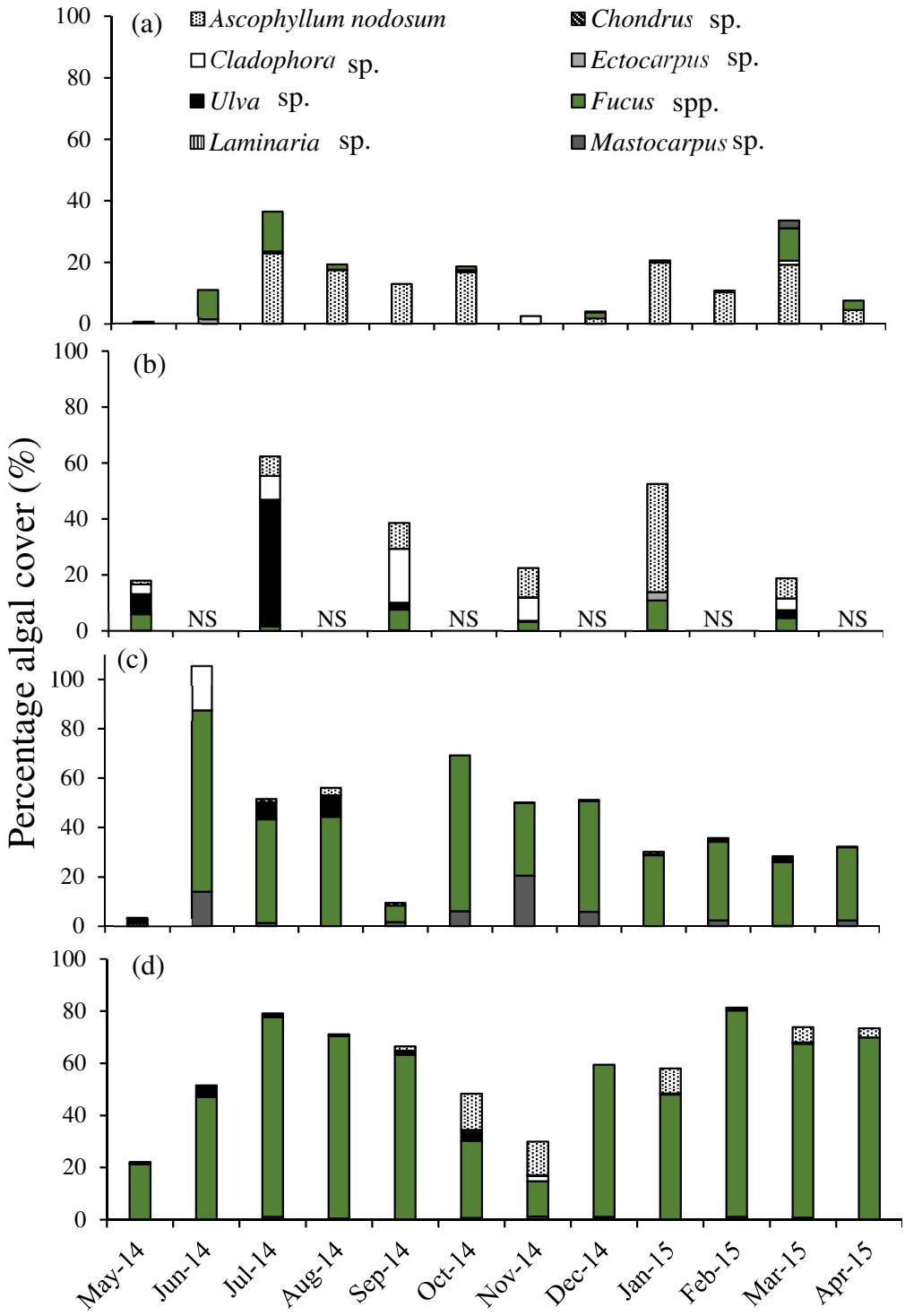

Fig. 1 Averaged percentage cover of macroalgal taxa recorded per sampling event per site (a) Cusheen, (b) Lough Hyne (c) Sandycove and (d) Whitegate. NS represent not sampled as Lough Hyne was only sampled & surveyed every second month.

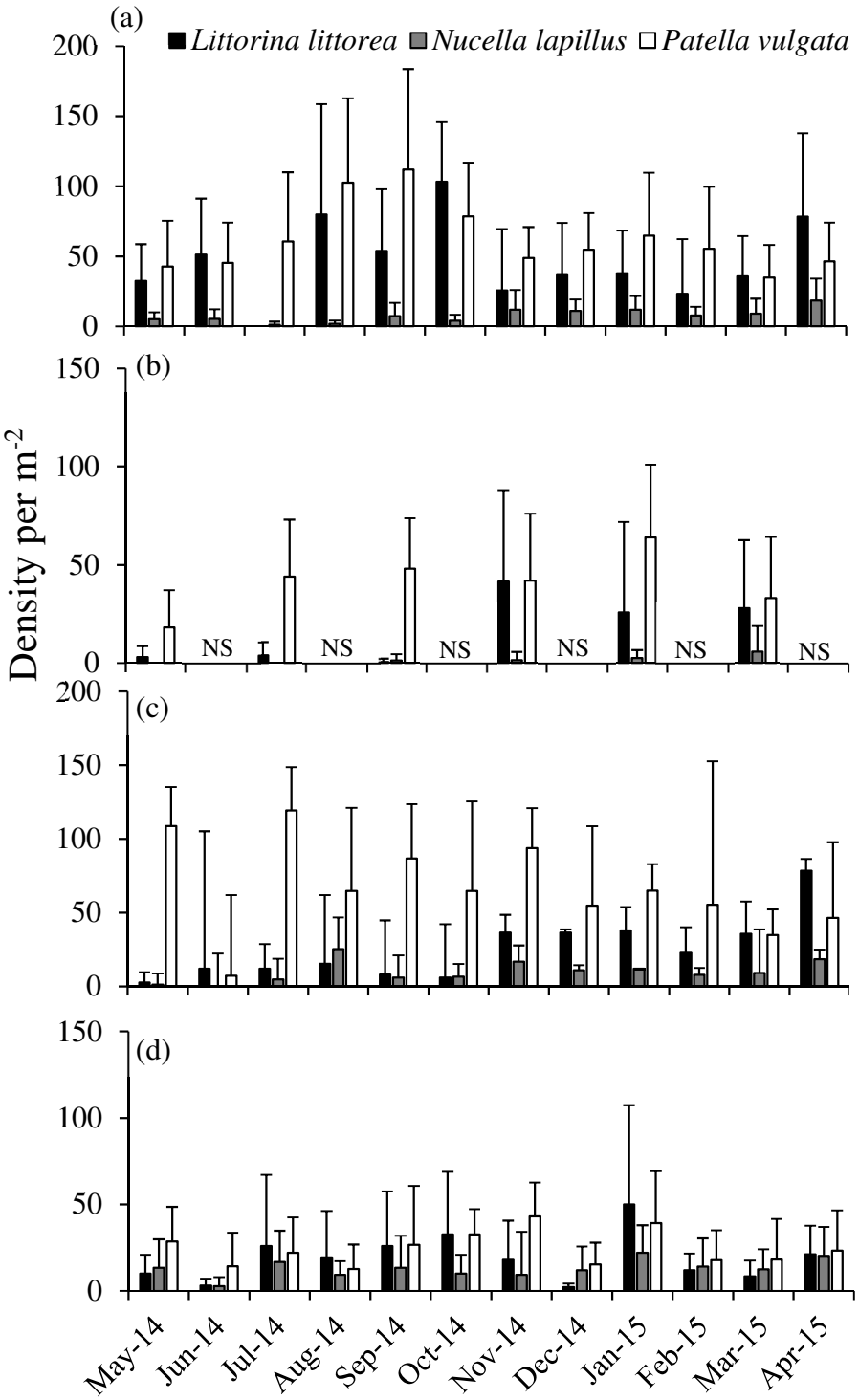

Fig. 2 Averaged density of gastropod host species screened taxa recorded per sampling event per site (a) Cusheen, (b) Lough Hyne, (c) Sandycove and (d) Whitegate. NS represent not sampled as Lough Hyne was only sampled & surveyed every second month. Note the differing y-axes

Table 1 Comparison of collection methods of host species on sample morphometrics and parasite prevalence. Asterisk denote level of significant P-values *<0.05, **<0.001, ***<0.0001.

Location	Collection method	Shell height (mm)	Trematode (%)	Polychaete (%)	Cestode (%)	Ciliate (%)	Mite (%)
Whitegate	Hand	22.97 ±3.90	6.67	3.33	NA	3.33	0.00
	QR	22.74±4.39	5.71	8.57	NA	0.00	0.00
	QR	21.28±5.81	4.00	4.00	NA	4.00	0.00
	QT	20.81±5.28	11.76	2.94	NA	5.88	0.00
Sandycove	QR	21.51±8.91	11.11	0.00	NA	0.00	0.00
	QT	21.56±3.79	5.88	0.00	NA	0.00	2.94
Whitegate	QR	24.36±5.74	0.00	0.00	NA	0.00	0.00
	QT	28.13±3.95**[1]	2.78	0.00	NA	0.00	0.00
Tralispean	QR	12.79±2.96*[2]	15.63	0.00	15.63	0.00	0.00
	QT	11.23±4.52	33.33	0.00	2.56	0.00	0.00
Sandycove	Hand	15.40±2.99**[3]	83.33*[4]	20.00	15.63	33.33	6.67
	QR	12.97±5.30	53.33	43.33	33.33	56.67	3.33

[1] F=7.86, df=1, P<0.01
[2] F=4.67, df=1, P<0.05
[3] F=8.76, df=1, P<0.01
[4] χ^2=4.93, df=1, P<0.05

Table 2 Prevalence of trematode taxa in foot tissues of *Mytilus* spp. screened during in the three trials run in Chapter 5. Prevalence of *Echinostephilla patellae* is excluded from the table as in the trials *Mytilus* spp. were exposed to *E. patellae*. – or + indicate whether *Mytilus* spp. was exposed to *Echinostephilla patellae* cercariae. For Trial 1, I represent intertidal and S subtidal *Mytilus* spp. collected in Lough Hyne. For Trial 2, Cu represents Cusheen, LH Lough Hyne, R Ringaskiddy. For Trial 3, C represents the control, L low nutrient enrichment, M medium enrichment, and H High enrichment treatments.

Trial	Treatment	Foot tissue		
		Renicola spp.	*Himasthla* spp.	*Echinostephilla patellae*
1	I-	0.00	0.00	58.33 (1-191)
1	I+	0.00	0.00	66.67 (2-120)
1	S-	0.00	0.00	8.33 (22)
1	S+	0.00	0.00	33.33 (1-5)
2	Cu-	33.33 (1-2)	88.89 (1-13)	33.33 (1-2)
2	Cu+	11.11 (1)	77.78 (1-9)	11.11 (1)
2	LH-	22.22 (1)	44.44 (2-19)	22.22 (1)
2	LH+	11.11 (1)	66.67 (3-25)	11.11 (1)
2	R-	0.00	11.11 (2)	0.00
2	R+	22.22 (1)	33.33 (1-9)	22.22 (1)
3	C-	29.41 (1-4)	41.18 (1-10)	5.88 (1)
3	C+	50.00 (1-3)	18.75 (2-4)	6.25 (1)
3	L-	42.86 (1-2)	57.14 (1-10)	14.29 (1-3)
3	L+	47.06 (1-5)	70.59 (1-7)	0.00
3	M-	37.50 (1-4)	43.75 (1-37)	12.50 (1-5)
3	M+	43.75 (1-6)	62.50 (1-7)	6.25 (1)
3	H-	33.33 (1-4)	33.33 (1-4)	0.00
3	H+	41.18 (1-7)	5.88 (1)	11.76 (1-5)
Grand Total		**26.52 (1-7)**	**35.65 (1-37)**	**16.52 (1-191)**

Table 3 Overall prevalence and range in intensity of trematode taxa recorded in host taxa in the thesis. The life cycle stage of trematode taxa are listed in order of infection i.e., sporocyst, metacercaria and adult worm. TC or H indicates the method of detection was either tissue compression or histological. Host taxa habitat is classified as rocky shore intertidal (RI), rocky shore subtidal (RS) or as estuarine intertidal (EI). Trematode taxa are spilt relative to their lifecycle stages due to their differing routes of transmission and effects on host taxa. *Mytilus* spp.* represent individuals not included in trials in Chapter 5.

Host taxa	Trematode taxa	Total prevalence (%)	Range of Intensity	Method	Host taxa habitat	Estimated from Chapter
SPOROCYST						
(Primary intermediate hosts)						
Littorina littorea	*Paramonostomum chabaudi*	0.20 (n=1, 572)	NA	TC	RI	2
	Cryptocotyle lingua	1.63	NA	TC	RI	2
	Himasthla spp.	0.27	NA	TC	RI	2
	Podocotyle atomon	1.56	NA	TC	RI	2
	Renicola spp.	0.07	NA	TC	RI	2
Mytilus spp.	Trematode taxa (likely *Bucephalus* spp.)	0.14 (n=731)	NA	H	RS	5
Nucella lapillus	*Parorchis acanthus*	0.10 (n=956)	NA	TC	RI	2
METACERCARIA						
(Second intermediate hosts)						
Carcinus maenas	*Microphallus* spp.	86.90 (n=290)	1-2, 436	TC	EI	4
Hediste diversicolor	Echinostomid	15.41 (n=713)	1-13	TC	EI	4
	Gymnophallus spp.	5.44	1-19	TC	EI	4
L. littorea	*Renicola* spp.	7.13	1-875	TC	RI	2
	Echinostephilla patellae	0.68	1	TC	RI	2
	Himasthla spp.	0.07	1	TC	RI	2

Table 3 Continued

Host taxa	Trematode taxa	Total prevalence (%)	Range of Intensity	Method	Host taxa habitat	Estimated from Chapter
Mytilus spp.	Trematode taxa	3.53 (n=731)	1-11	H	RS	5
Mytilus spp.*	*E. patellae*	15.20 (n=125)	1-16	TC	RI	5
	Himasthla spp.	37.60	1-56	TC	RI	5
	Renicola spp.	77.60	1-123	TC	RI	5
		10.00 (n=10)	1	TC	RS	5
N. lapillus	*Renicola* spp.	1.15	1-12	TC	RI	2
	E. patellae	0.31	1-4	TC	RI	2
Patella vulgata	*E. patellae* (n=1,745)	40.90	1-223	TC	RI	2 & 3
	Gymnophallus spp.	0.23	1-103	TC	RI	2 & 3
	Renicola spp.	0.28	1-8	TC	RI	2
ADULT WORM (Final hosts)						
N. lapillus	*Proctoeces* sp.	0.10	1	TC	RI	2
P. vulgata		0.07	1	TC	RI	2

Table 4 Summary of lifecycles of trematode taxa recorded during this study. All chapters and sampling events combined.

Family	Genera	First intermediate host	Second intermediate host	Final host	Free living life stages	Reference
Echinostomatidae	*Himasthla* sp.	Gastropods *Littorina littorea*	Molluscs & polychaetes *L. littorea*	Birds	2	James 1968a
	Himasthla militaris	Gastropods	Polychaetes *Hediste diversicolor*	*Larus glaucescens, Arenaria interpres, Calidris maritime, S. mollissima, Chen canagica, Motacilla alba*	2	Timon-David & Rebecq 1959, Loos-Frank, 1967, Vanoverschelde & Vaes 1980
Fellodistomidae	*Proctoeces* sp.	Gastropods	Gastropods	Gastropods *Patella vulgata* *Nucella lapillus*	1	Stunkard & Uzmann 1959, Lauckner, 1980
Gymnophallidae	*Gymnophallus* sp.	Gastropods	Gastropods & Bivalves *Patella vulgata*	Birds	2	Stunkard & Uzmann 1958, Lauckner, 1980
	Gymnophallus choledochus	Gastropods	Molluscs & polychaetes	Charadriiform, lariform and anatid birds.		Loos-Frank 1969
Heterophyidae	*Cryptocotyle lingua*	Gastropods *Littorina littorea*	Bony fish	Birds and mammals	2	Stunkard 1930, James 1968a

Table 4 Continued

Family	Genera	First intermediate host	Second intermediate host	Final host	Free living life stages	Reference
Microphallidae	*Microphallus primas*	Gastropods	Crustaceans Carcinus maenas	Birds Charadriiformes and Anseriformes	2	Biguet *et al.,* 1958, Saville & Irwin 1991
Notocotylidae	*Paramonostomum chabaudi*	Gastropods *Littorina littorea*	Vegetation or substrate	Birds	1	James 1968, Evans *et al.,* 1997
Opecoelidae	*Podocotyle atomon*	Gastropods *L. littorea*	Benthic crustaceans, mainly amphipods	Teleosts	2	James 1968a, Thieltges & Reise 2007
Philophthalmidae	*Echinostephilla patellae*	Gastropods *P. vulgata*	Gastropods and Bivalves *Mytilus* spp. *P. vulgata*	Wading birds	2	Kollien, 1996, Prinz *et al.,* 2010b
	Parorchis acanthus	Gastropods *Nucella lapillus*	Gastropods	Seabirds	2	Yamaguti 1971, Lauckner, 1980,
Renicolidae	*Renicola* spp.	Gastropods *L. littorea*	Molluscs *L. littorea*	Fish consuming birds	2	Stunkard & Uzmann 1959, James 1968, Lauckner, 1980

Table 4 Continued

Family	Genera	First intermediate host	Second intermediate host	Final host	Free living life stages	Reference
Echinostomatidae	*Himasthla* sp.	Gastropods *Littorina littorea*	Molluscs & polychaetes	Birds	2	James 1968a
	Himasthla militaris	Gastropods	Polychaetes *Hediste diversicolor*	*Larus glaucescens, Arenaria interpres, Calidris maritime, S. mollissima, Chen canagica, Motacilla alba*	2	Timon-David & Rebecq 1959, Loos-Frank, 1967, Vanoverschelde & Vaes 1980
Fellodistomidae	*Proctoeces* sp.	Gastropods	Gastropods	Gastropods *Patella vulgata* *Nucella lapillus*	1	Stunkard & Uzmann 1959, Lauckner, 1980
Gymnophallidae	*Gymnophallus* sp.	Gastropods	Gastropods & Bivalves *Patella vulgata*	Birds	2	Stunkard & Uzmann 1958, Lauckner, 1980
	Gymnophallus choledochus	Gastropods				Loos-Frank 1969
	Cryptocotyle lingua	Gastropods *Littorina littorea*	Bony fish	Birds and mammals	2	Stunkard 1930, James 1968a

Table 4 Continued

Family	Genera	First intermediate host	Second intermediate host	Final host	Free living life stages	Reference
Microphallidae	*Microphallus primas*	Gastropods	Crustaceans *Carcinus maenas*	Birds Charadriiformes and Anseriformes	2	Biguet *et al.,* 1958, Saville & Irwin 1991
Notocotylidae	*Paramonostomum chabaudi*	Gastropods *Littorina littorea*	Vegetation or substrate	Birds	1	James 1968, Evans *et al.,* 1997
Opecoelidae	*Podocotyle atomon*	Gastropods *L. littorea*	Benthic crustaceans, mainly amphipods	Teleosts	2	James 1968, Thieltges & Reise 2007
Philophthalmidae	*Echinostephilla patellae*	Gastropods *P. vulgata*	Gastropods and Bivalves *Mytilus* spp. *P. vulgata*	Wading birds	2	Kollien, 1996, Prinz *et al.,* 2010b
	Parorchis acanthus	Gastropods *Nucella lapillus*	Gastropods	Seabirds	2	Yamaguti 1971, Lauckner, 1980,
Renicolidae	*Renicola roscovita*	Gastropods *L. littorea*	Molluscs *L. littorea*	Fish consuming birds	2	Stunkard & Uzmann 1959, James 1968, Lauckner, 1980